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The effect of blood glucose on the vasculature in young patients with type 1 diabetes

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Academic dissertation
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Contents

Abstract

Background. Patients with type 1 diabetes are at markedly increased risk for vascular complications. In this respect it is noteworthy that hyperglycaemia, shown to cause endothelial dysfunction, has clearly been shown to be a risk factor for diabetic microvascular disease. The role of hyperglycaemia as a predictor of macrovascular disease is, however, not as clear as for microvascular disease, although type 1 diabetes itself elevates risk for cardiovascular disease substantially. Furthermore, what is unknown is whether the short-term or the long-term hyperglycaemia confers the possible risk. In addition, the role of glucose variability as a predictor of complications is to a large extent unexplored. Interestingly, although hyperglycaemia elevates risk for pre-eclampsia in women with type 1 diabetes, it is unclear whether pre-eclampsia, a condition characterized by endothelial dysfunction, is also a risk factor for microvascular complication, diabetic nephropathy.

Aims. This doctoral thesis investigates the role of acute hyperglycaemia and glucose variability on arterial stiffness and cardiac ventricular repolarisation in male patients with type 1 diabetes as well as in healthy male volunteers. It also explores whether acute hyperglycaemia leads to an inflammatory response, endothelial dysfunction, and oxidative stress. Finally, the role of pre-eclampsia, as a predictor of diabetic nephropathy in type 1 diabetes is examined.

Subjects and methods. In order to study glucose variability and daily glycaemic control, 22 male patients with type 1 diabetes, but without any diabetic complications, were monitored for 72 h with a continuous glucose monitoring system. At the end of the 72 h glucose monitoring period a 2 h hyperglycaemic clamp was performed both in the patients with type 1 diabetes and in the 13 healthy age-matched male volunteers. Blood pressure, arterial stiffness, and QT time were measured to detect vascular changes during acute hyperglycaemia. Blood samples were drawn at baseline (normoglycaemia) and during acute hyperglycaemia. In another patient sample, women with type 1 diabetes were followed during their pregnancy and restudied 11 years later to elucidate the role of pre-eclampsia and pregnancy-induced hypertension as potential risk factors for diabetic nephropathy.

Results and conclusions. Acute hyperglycaemia increased arterial stiffness as well as caused a disturbance in the myocardial ventricular repolarisation, emphasizing the importance of strict daily glycaemic control in male patients with type 1 diabetes. An inflammatory response was also observable during acute hyperglycaemia. Furthermore, high mean daily blood glucose but not glucose variability *per se* is associated with arterial stiffness. While glucose variability in turn correlated with central blood pressure, the results suggest that the glucose metabolism is closely linked to the haemodynamic changes in male patients with uncomplicated type 1 diabetes. Notably, these results are not directly applicable to females. Finally, a history of a pre-eclamptic pregnancy, but not pregnancy-induced hypertension was associated with increased risk for diabetic nephropathy.

List of original publications

This thesis is based on the following publications:

I Gordin D, Rönnback M, Forsblom C, Heikkilä O, Saraheimo M, Groop P-H. Acute hyperglycaemia rapidly increases arterial stiffness in young patients with type 1 diabetes. *Diabetologia* 2007;50:516-22.

II Gordin D, Rönnback M, Forsblom C, Mäkinen V, Saraheimo M, Groop P-H. Glucose variability, blood pressure and arterial stiffness in type 1 diabetes. *Diabetes Research and Clinical Practice* 2008;80:e4-7.

III Gordin D, Forsblom C, Rönnback M, Groop P-H. Acute hyperglycaemia disturbs cardiac repolarization in type 1 diabetes. *Diabetic Medicine* 2008;25:101-5.

IV Gordin D, Forsblom C, Rönnback M, Parkkonen M, Wadén J, Hietala K, Groop P-H. Acute hyperglycaemia induces an inflammatory response in young patients with type 1 diabetes. *Annals of Medicine* 2008;16:1-7.

V Gordin D, Hiilesmaa V, Fagerudd J, Rönnback M, Forsblom C, Kaaja R, Teramo K, Groop P-H, Finn-Diane Study Group. Preeclampsia but not pregnancy induced hypertension is a risk factor for diabetic nephropathy in type 1 diabetes. *Diabetologia* 2007;50:516-22.

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Abbreviations

ACE	angiotensin-converting enzyme
ADA	American Diabetes Association
AER	albumin excretion rate
AIx	augmentation index
AMI	acute myocardial infarction
ARB	angiotensin receptor-blocking agent
BMI	body mass index
CGMS	continuous glucose monitoring system
CRP	C-reactive protein
DBP	diastolic blood pressure
DCCT	Diabetes Control and Complications Trial
DN	diabetic nephropathy
EASD	European Association for the Study of Diabetes
EDIC	Epidemiology of Diabetes Interventions and Complications
ET-1	endothelin-1
$HbA1_c$	glycated haemoglobin A_{1c}
HDL	high-density lipoprotein
IL-6	inteleukin-6
ICAM-1	intracellular adhesion molecule-1
LDL	low-density lipoprotein
MAGE	mean amplitude of glycaemic excursions
PE	pre-eclampsia
PIH	pregnancy-induced hypertension
PP	pulse pressure
PWA	pulse wave analysis
PWV	pulse wave velocity
QTc	QT interval corrected for heart rate
T1D	type 1 diabetes
TNF-α	tumour necrosis factor- α
SBP	systolic blood pressure
SMBG	self-monitoring blood glucose
SOD	superoxide dismutase
UKPDS	United Kingdom Prospective Diabetes Study
VCAM-1	vascular adhesion molecule-1

1 Introduction

The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that tight glycaemic control is important in order to prevent diabetic microvascular complications [1] [2]. Conversely, chronic hyperglycaemia has not unequivocally been proven to be a risk factor for macrovascular complications [3], although diabetes itself leads to increase risk for cardiovascular disease [4] [5].

The possible role played by acute change in glycaemic control as a risk factor for diabetic complications is poorly understood. It is, however, noteworthy that acute hyperglycaemia seems to be a risk factor for worse prognosis during acute cardiovascular events in patients with and without diabetes [6]. Postprandial hyperglycaemia is, in turn, a risk factor for cardiovascular disease in patients with type 2 diabetes [7]. Acute hyperglycaemia is also suggested to cause vascular changes in non-diabetic subjects [8]. Nevertheless, whether acute hyperglycaemia affects the vasculature in patients with type 1 diabetes is largely unknown.

Surprisingly, despite the fact that patients with type 1 diabetes are known to show marked glucose fluctuations on a daily basis, glucose variability in relation to diabetic complications is a less studied subject in vivo. A recent study has, however, shown that glucose variability is a risk factor for patients in intensive care [9]. In contrast, a few recent studies have suggested that glucose variability does not predict diabetic complications [10] [11]. Any potential association between glucose variability and macrovascular disease in patients with type 1 diabetes is incompletely explored.

Intermediate markers are widely used in patients with diabetes to predict complications. Arterial stiffness is one such factor with important clinical consequences and may serve as an intermediate marker for cardiovascular disease. Furthermore, heart rate-corrected QT time (QTc) is a measure of cardiac ventricular repolarisation. A prolonged QTc is an established risk factor for cardiac sudden death and hence suitable as an intermediate marker for macrovascular complications [12].

Chronically elevated blood glucose concentrations are thought to cause diabetic complications through a number of different mechanisms such as chronic inflammation, endothelial dysfunction, and oxidative stress, and these features have also been linked to both micro- and macrovascular complications [13]. However, it is unclear whether acute hyperglycaemia also leads to an inflammatory response, endothelial dysfunction, and oxidative stress and subsequent vascular changes in patients with type 1 diabetes, although for patients with type 2 diabetes such data exist [14].

Pre-eclampsia is a serious condition that affects a proportion of pregnant women. It is characterized by endothelial dysfunction, hypertension, and massive proteinuria. Hyperglycaemia is a risk factor for pre-eclampsia, and the incidence of the disorder is markedly increased in patients with type 1 diabetes [15]. Diabetic nephropathy is, in turn, a serious microvascular complication of diabetes. Although pre-eclampsia and nephropathy have many common pathogenic factors, no studies have

elucidated whether pre-eclampsia is a predictor of diabetic nephropathy in patients with type 1 diabetes.

2 Review of the literature

2.1 Type 1 diabetes

An estimated 246 million people worldwide have diabetes [16]. Among all patients suffering from this disease, type 1 diabetes accounts for 5 to 10% [17]. The incidence is for mostly unknown reasons the highest in the world in Finland [18], and the condition substantially influences patients' daily life and prognosis [19]. Type 1 diabetes is caused by an autoimmune destruction of the insulin-producing pancreatic beta-cells that eventually leads to a total loss of insulin secretion. The disease usually emerges acutely and is characterized by hyperglycaemia, ketoacidosis, polyuria, weight loss and dehydration. A life-saving treatment was discovered in the 1920's by Banting and Best when they developed insulin therapy [20].

The etiology of type 1 diabetes is thought to involve both genetic and environmental factors. The genetic component is supported by the observation that the concordance rate of the disease varies from 20% to at least 50% for monozygotic twins, while the risk for a first-degree relative is approximately 5% [21 22 23]. An important genetic determinant that affects susceptibility lies within the major histocompatibility complex, although other locations are also intensively studied [24].

Although there is no doubt that environmental factors contribute to the development of type 1 diabetes, their true role in its pathogenesis is still a matter of debate. There exist, however, a few plausible hypotheses. Firstly, a viral component is proposed by the fact that the incidence is higher during certain periods of the year [25]. Secondly, some data suggest that the intake of bovine milk at an early age or even vaccinations might contribute to the risk, although these theories have also been challenged [26]. Thirdly, some results indicate that the environment in the western world may be too sterile and may thus lead to a weakening of the immunological mechanisms [27].

2.2 Long-term complications in type 1 diabetes

Long-term complications of diabetes are categorized into micro- and macrovascular disease. Diabetic microvascular complications consist of diabetic nephropathy, retinopathy, and neuropathy. Macrovascular disease denotes cardiovascular disease, cerebrovascular disease, and peripheral arterial disease.

2.2.1 Microvascular disease

An important risk factor for the development of diabetic microvascular disease is hyperglycaemia, a fact that has been shown in many large studies [1] [28] [29] [30]. Other risk factors also emerge, however, such as hypertension, smoking, dyslipidaemia, duration of diabetes, and genetic susceptibility [31] [32].

2.2.1.1 Diabetic nephropathy

Diabetic nephropathy is characterized by an increase in urinary albumin excretion rate (AER), elevated blood pressure, a relentless decline in renal function, endothelial dysfunction, and a 37-fold increased risk for cardiovascular mortality [33]. Patients with diabetic nephropathy compared to patients without have a more than 10-fold increased risk for developing cardiovascular disease [34].

Approximately one third of patients with type 1 diabetes will develop diabetic nephropathy, but recent data indicate that the proportion may be lower due to successful treatment of hyperglycaemia and hypertension [35] [36]. Poor glycaemic control, smoking, male gender, hypertension, and predisposing genes are risk factors for nephropathy in these patients [37] [38] [39]. Importantly, several detectable steps help the clinician make the diagnosis of diabetic nephropathy, because development of this renal complication goes through various stages, from microalbuminuria (defined as AER between ≥20 <200 µg/min or ≥30 <300 mg/24h), to overt nephropathy or macroalbuminuria (AER ≥200 µg/min or ≥300 mg/24h) and finally leads to end-stage renal disease. Given the grim statistics regarding the prognosis of patients with diabetic nephropathy, efficient screening for microalbuminuria is a cornerstone of the management of patients with type 1 diabetes. Patients should therefore be screened for microalbuminuria annually starting 5 years after the diagnosis of type 1 diabetes.

The importance of strict glycaemic control cannot be overemphasized for the treatment of type 1 diabetes as a means to avoid complications. However, at the stage of advanced diabetic nephropathy, the role and efficacy of strict glycaemic control is less clear. In contrast, angiotensin-converting enzyme inhibitors and angiotensin receptor-blocking agents are drugs of choice not only to lower blood pressure but also to protect the kidneys at all stages of diabetic nephropathy. Lipid control and cessation of smoking are also of importance, alongside the administration of renoprotective agents [40]. Notably, the treatment is not only essential to protect the kidneys from damage but also essential because microalbuminuria is an independent predictor of cardiovascular disease [41].

2.2.1.2 Diabetic retinopathy

Diabetic retinopathy is an important microvascular complication in diabetes in the western world and a major cause of blindness [42]. The natural cause or the progression of the complication can be divided into a number of clinically detectable stages. It starts with non-proliferative changes

(microaneurysms, exudates, haemorrhages), and advances to preproliferative retinopathy, proliferative retinopathy, and macular oedema.

After 20 years of diabetes almost all patients with type 1 diabetes show signs of retinopathy. Most of the patients have background retinopathy, a complication that seldom leads to severe vision loss. However, when retinopathy worsens, severe visual loss eventually threatens 5 to 10% [43]. The most severe form of retinopathy is proliferative retinopathy and most with this complication will become blind after 5 to 10 years without treatment [44]. After 15 to 20 years of diabetes the prevalence of proliferative retinopathy ranges from 13 to 50%[45].

Hyperglycaemia, hypertension, microalbuminuria, dyslipidaemia, and duration of diabetes are all risk factors for diabetic retinopathy [46] [47]. Notably, a strong correlation emerges between diabetic nephropathy and proliferative retinopathy [48]. All patients with type 1 diabetes should undergo regular screening to detect retinopathy. The prevention and treatment of diabetic retinopathy includes strict glycaemic control, antihypertensive treatment, and lipid control; laser therapy is an effective means to avoid blindness [49].

2.2.1.3 Diabetic neuropathy

Diabetic neuropathy is a common long-term complication of diabetes, affecting some 50% of patients [50]. It can be divided into two major forms: generalized and focal [51]. A common form of generalized neuropathy is peripheral sensorimotor polyneuropathy. Peripheral polyneuropathy together with a poor peripheral arterial circulation often results in problems with wound healing, gangrene and in the worst case, limb amputation. The generalized diabetic neuropathy also includes autonomic neuropathy, seen often as cardiac dysfunction, exercise intolerance, gastroparesis, or erectile dysfunction. Typical focal neuropathies include carpal tunnel syndrome, diabetic amyotrophy, and nerve palsies [52].

Physicians need to screen for neuropathy starting 5 years after the diagnosis of diabetes, and this screening should be carried out on a regular basis with appropriate methods. Again, tight glycaemic control is the key player in the prevention and management of diabetic neuropathy [53]. Tricyclic agents are used to treat painful neuropathy, and anticonvulsants or even opioids are the choice in some cases to manage severe pain that does not respond to other treatment [54].

2.2.2 Macrovascular disease

Type 1 diabetes elevates the risk for atherosclerosis and macrovascular disease substantially but despite the fact that these complications are rather common, the pathogenesis is still poorly understood. Risk for cardiovascular disease has been more than 10-fold higher in patients with diabetic nephropathy [34], but even patients without nephropathy still are at increased risk [55] [56] [57]. The classic risk factors for atherosclerosis such as smoking, high blood pressure, dyslipidaemia, age, and impaired glucose tolerance seem to be operative also in this patient group, although they cannot

alone explain the increased risk. Hyperglycaemia would be a plausible factor to serve as the logical missing link, but it has, however, not unequivocally been shown to be a risk factor for macrovascular complications [3 58 59].

Recent data from the DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC) study showed that intensive insulin therapy with subsequent tight glycaemic control reduces risk for cardiovascular disease in patients with type 1 diabetes [60]. It is, however, of note that only 83 of the 1441 patients studied suffered cardiovascular events. Moreover, the EDIC reported a lower rate of progression of carotid intima-media thickening and reduced coronary artery calcification as surrogate markers of cerebrovascular and cardiovascular disease in the patient group with better glycaemic control [61 62]. Similar results are also available regarding peripheral arterial disease [63]. Interestingly, the Pittsburgh Epidemiology of Diabetes Complications Study (EDC) reported a stronger correlation between glycaemia and cardiovascular disease in patients with normoalbuminuria than in patients with diabetic nephropathy [64]. In addition, results from the EURODIAB Study showed that HbA_{1c} was related to coronary heart disease in men but not in women with type 1 diabetes [65].

Regarding the role of chronic hyperglycaemia as a risk factor for cardiovascular disease in patients with type 2 diabetes, data are conflicting. The UKPDS did not exhibit a reduction in cardiovascular events in patients with type 2 diabetes, although a subgroup of patients treated with metformin had a lower risk for such events [66]. Intriguingly, two large trials, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial and the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial, failed to demonstrate any effect on cardiovascular risk in patients with type 2 diabetes from lowering glucose to near-normal levels [67 68]. In fact, the near-normal glucose control in the ACCORD trial was associated with significantly increased risk of death from any cause and death from cardiovascular disease.

Although the data are still somewhat conflicting, glucose control is suggested to be important in preventing not only microvascular but also macrovascular disease [60]. Moreover, the recommendations for lipid control are even more strict for patients with type 1 diabetes than for the non-diabetic population [69 70]. Strict control of blood pressure (<130/80 mmHg) is also recommended by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) [71 72]. The drugs of choice for patients with diabetes are either angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor-blocking (ARB) agents. However, the antihypertensive medication should always be individually tailored for the patient, and other possible diseases (for example beta-blockers in cases of coronary heart disease) should also be taken into account. Aspirin therapy should be initiated for all patients with type 1 diabetes >40 years of age or who have at least one cardiovascular disease risk factor [70]. Cessation of smoking is of outmost importance for the prevention of cardiovascular disease [73].

2.3 Hyperglycaemia

The blood glucose concentration in a non-diabetic individual is strictly controlled by the interplay between a number of hormones, physiological insulin secretion from the pancreas, and insulin action in the target organ, as well as glucagon, catecholamines, cortisone, growth hormones, and prolactin. As the insulin secretion fails in patients with type 1 diabetes, insulin has to be administered exogenously for the patient to stay alive. However, modern insulin regimens aim not only to compensate for the insulin depletion but also to optimize glycaemic control and to minimize the blood glucose variability.

2.3.1 Acute hyperglycaemia

Blood glucose is usually measured by the patients themselves by self-monitoring blood glucose (SMBG) devices. The recommendations suggest three or more SMBG measures daily to achieve better glycaemic control [70]. Blood glucose can also be measured by a continuous glucose monitoring system (CGMS). Acute hyperglycaemia can lead to polyuria, dehydration, and ketosis, whereas acute hypoglycaemia can lead to cold sweating, tachycardia, and if severe even to loss of consciousness.

Acute hyperglycaemia is associated with increased acute cardiovascular mortality, whether the patient has diabetes or not [6]. Moreover, evidence exists that patients with hyperglycaemia during severe stress such as traumas have a worse prognosis than normoglycaemic patients [74].

The effect of acute hyperglycaemia on the cardiovascular system is not completely known. However, acute hyperglycaemia has been demonstrated to cause arrhythmias in patients with acute myocardial infarction (AMI) [75]. This is in line with another important finding that an acutely increased blood glucose concentration will prolong the QT time in healthy volunteers [76]. This observation has been repeated in a rat model [77]. In addition, acutely increased blood glucose concentrations are associated with impaired left ventricular function, and a larger size infarction in patients with an AMI [78] [79]. Importantly, Mullan et al. reported significant arterial stiffening during acute hyperglycaemia in healthy males [8]. Such data may explain the enhanced mortality in association with an AMI in patients with acute hyperglycaemia.

As to changes in haemostasis, evidence suggests an activation of the blood coagulation system during acute hyperglycaemia [80]. It is noteworthy that Oswald et al. some 20 years ago, had already showed an association between thrombophilia and hyperglycaemia in non-diabetic patients with an AMI [81]. Taken together, all these findings may explain the increased risk of mortality during acute cardiovascular events in patients with high blood glucose concentrations.

2.3.1.1 Inflammation

Chronic inflammation has during recent years been linked to cardiovascular disease: atherosclerosis and acute coronary syndromes [82] [83]. Some data also link worse cardiac outcome and inflammatory markers in patients with AMI [84]. Moreover, highly sensitive C-reactive protein (CRP), and interleukin-6 (Il-6) independently predict the outcome of patients with coronary heart disease in the general population [85] [86]. Ceriello and his colleagues have shown that acute hyperglycaemia enhances the production of inflammatory markers in healthy subjects, in patients with impaired glucose tolerance, and in patients with type 2 diabetes [87] [88] [89]. Whether an acute rise in blood glucose triggers an inflammatory response in patients with type 1 diabetes is not known

2.3.1.2 Endothelial function

The endothelium is the organ forming the inner layer of the vasculature, and given its widespread distribution all over the body and its multi-faceted secretion of vasoactive substances, it is also considered the largest endocrine organ. The endothelium has a number of vital functions. Its morphology and function differ throughout the vasculature. It participates in thrombolysis, platelet adhesion, inflammatory processes, and substrate exchange, as well as in the regulation of vascular tone and growth [139].

It has also been shown to be involved in the pathogenesis of vascular disease. Several studies have consistently reported impaired endothelial function as a consequence of an increased blood glucose concentration [90] [91] [92]. Endothelial dysfunction is also thought to be involved in the formation of atherosclerotic plaques [93], and therefore, not surprisingly, is associated not only with AMI [94] but also with chronic inflammation [95]. Interestingly, a recent study suggested that in patients with type 1 diabetes a synchronous control of oxidative stress and hyperglycaemia normalizes the endothelial function [96].

2.3.1.3 Oxidative stress

Mitochondrial superoxide overproduction leads to tissue damage through reactive oxygen species. Oxidative stress is a well-known pathogenic factor for both atherosclerosis and cardiovascular disease [97]. Nitrotyrosine, a marker of oxidative stress, has been an independent predictor of cardiovascular disease [98]. It is noteworthy that acute hyperglycaemia has been linked to oxidative stress [99] not only in experimental animals but also in healthy subjects and patients with type 2 diabetes [100] [101] [102]. An acutely elevated blood glucose concentration has recently been shown to downregulate gene expression in adipose tissue and skeletal muscle in healthy subjects [103]. Finally, antioxidants can reduce the adverse effects of acute hyperglycaemia on endothelial dysfunction and inflammation [104].

2.3.1.4 Treatment of acute hyperglycaemia

The increased cardiovascular mortality in patients with acute hyperglycaemia argues for tight blood glucose control during an acute cardiovascular event. The Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) I study demonstrated that an insulin-glucose infusion followed by at least 3 months of multiple-dose insulin treatment was able to reduce mortality in patients with type 1 or type 2 diabetes [105] [106]. The DIGAMI II study could not, however, confirm these results [107]. Furthermore, the Leuven Study showed that intensive insulin therapy lowered both morbidity and mortality among critically ill patients with or without diabetes treated in a surgical intensive care unit [108]. Similar data are also available for patients treated in a medical intensive care unit [109]. But other data contradicts this [110].

These results started a debate as to whether the blood glucose control or the insulin itself had improved these patients' prognosis [111]. The debate is certainly relevant, since a beneficial vasodilatory effect of insulin on the vasculature has also been demonstrated [112]. Importantly, recent trials could show no decrease in short-term mortality of patients with AMI when glucose-insulin-potassium infusions were given acutely [113] [114]. More trials are needed to obtain answers to these open questions.

2.3.2 Chronic hyperglycaemia

Long-term glucose control has for more than two decades been assessed with the HbA_{1c} assay, a method that has also become the gold standard for the determination of chronic hyperglycaemia [115]. The results of the DCCT and other similar trials have established a relationship between HbA_{1c} and the risk for diabetic complications in patients with type 1 diabetes [1] [28] [29] [30] [116].

HbA_{1c} reflects average glycaemia over a period of a few months, explained by the fact that the lifespan of an erythrocyte containing haemoglobin is approximately 120 days [117]. However, no accurate studies to actually test this hypothesis have existed until now [118]. The ADA, the EASD, the International Diabetes Federation (IDF), and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) participated in the HbA_{1c}–derived average glucose (ADAG), study that explored the relationship between the daily average blood glucose measured by the CGMS and the HbA_{1c}, in order to be able to accurately standardize the HbA_{1c} measurement [119].

In the current recommendations for the treatment of patients with diabetes, the target level of HbA_{1c} is set at less than 7% [70]. Nevertheless, not even half the patients with diabetes have achieved or will achieve this goal [120]. Improvement of glucose control is a great challenge for any physician.

2.3.2.1 Inflammation

Chronic inflammation is suggested to explain at least in part the enhanced cardiovascular mortality of patients with type 1 diabetes [121] [122] [123]. It is therefore not surprising that inflammatory markers

such as CRP and Il-6 are elevated both in patients with type 2 diabetes and with cardiovascular disease [124] [125]. Growing evidence also suggests that that inflammatory markers are increased in patients with type 1 diabetes with and without microvascular disease [126] [127].

In addition, soluble intercellular adhesion molecule 1 (ICAM-1) and soluble tumor necrosis factor α (TNF-α) predict risk for cardiovascular disease in non-diabetic subjects [128] [129] and are increased in patients with type 1 and type 2 diabetes [130] [131]. Notably, a recent publication from the DCCT study demonstrated that tight glycaemic control reduced the concentrations of ICAM-1 [132].

2.3.2.2 Endothelial dysfunction

To what extent chronic hyperglycaemia affects endothelial function is not yet fully unraveled. Endothelial function cannot be measured directly in humans, but some information may be obtained indirectly by measuring endothelium-dependent and independent vasodilation, plasma levels of endothelium-derived regulatory proteins, or possibly microalbuminuria [133]. Measurement of arterial stiffness has also been considered an applicable method to measure endothelial function indirectly [134]. The number of methods available to measure endothelial function, however, make the interpretation and the comparison of results rather difficult and hazardous.

Patients with diabetic complications such as nephropathy, retinopathy, and atherosclerosis are known to have disturbed endothelial function [135] [136]. Altered endothelial function is also suggested to be related to increased mortality in patients with type 2 diabetes [137]. Furthermore, Elliot et al. demonstrated that endothelial dysfunction is present in patients with type 1 diabetes and microalbuminuria [138]. In contrast, it is not entirely clear whether patients with uncomplicated type 1 diabetes already show endothelial dysfunction. This may in part be due to the fact that autonomic dysfunction modulates results [139] [140] [141]. Consequently, the blood vessels of patients with type 1 diabetes may actually dilate, not constrict, and thus cause increased microvascular blood flow in response to chronic hyperglycaemia [142].

All in all, the exact pathogenic mechanisms for these findings remain unresolved, but hyperglycaemia may be a plausible trigger of endothelial dysfunction, as well as of chronic inflammation and oxidative stress and may thus serve as the mediator of the endothelial damage.

2.3.2.3 Oxidative stress

Another potential mechanism by which chronic hyperglycaemia could cause diabetic complications is through overproduction of superoxide and oxidative stress. Oxidative stress was linked to diabetes as early as in 1979 [143]. What has, however, been discussed is whether oxidative stress precedes the appearance of complications or whether it merely reflects the presence of complications [144]. A few studies have shown that in children and adolescents with type 1 diabetes, increased oxidative reactions are already present [145] [146], although conflicting data are also available

[147]. At least, superoxide production is suggested to decrease in response to improved glycaemic control in adult patients with type 1 diabetes [148].

Four major molecular mechanisms have been implicated in glucose-mediated vascular damage. All seem to reflect hyperglycaemia-induced overproduction of superoxide by the mitochondrial electron-transport chain. Excess superoxide partially inhibits the glycolytic enzyme GADPH, and thereby diverts upstream metabolites (glucose, fructose-6-phosphate, glyceraldehyde-3-phosphate) from glycolysis to the following pathways: the polyol pathway, the hexosamine pathway, the protein kinase C (PKC) pathway, and the nonenzymatic protein glycation (AGE) pathway [149] [13] (Figure 1).

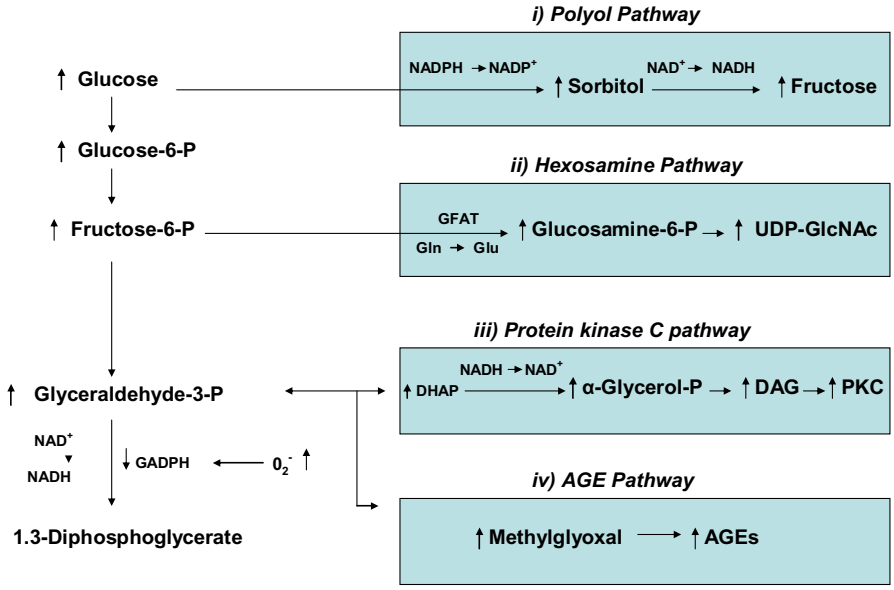


Figure 1. Four major pathways of hyperglycaemic damage mediated by oxidative stress. Adapted by permission from Macmillan Publishers Ltd: Nature. Brownlee M et al. 2001;414(6865):813-820, copyright 2001. www.nature.com.

In the polyol pathway, the enzyme aldose reductase is the key player. This enzyme normally catalyses the NADPH-dependent reduction of toxic agents in the cell, but when the intracellular glucose concentration is elevated, aldose reductase instead turns glucose into fructose. This next reduces the NADPH available for glutathione reductase, and since NADPH is required to regenerate reduced glutathione, reduced NADPH may lead to increased intracellular oxidative stress [150] [151].

The hexosamine pathway is also activated by a high intracellular glucose concentration. The fructose 6-phosphate is diverted from the glycolysis and then converted into glucosamine 6-

phosphate by the enzyme GFAT (glutamine:fructose-6-phosphate amidotransferase) and finally to UDP *N*-acetyl glucosamine. These biochemical changes are thought to disturb, among others, the function of cardiomyocytes in the heart [152].

In the protein kinase C pathway intracellular hyperglycaemia raises the amount of diacylglycerol (DAG), and DAG then activates PKC. PKC exerts a variety of effects on gene expression. It for example reduces the production of endothelial nitric oxide (NO) and raises the production of ET-1. It may also lead to increased capillary occlusion, angiogenesis, or inflammation [153] [154] [155]. Experimental animal models show that inhibition of PKC-β prevents alterations in the diabetic eye and kidney [156]. However, in vivo studies blocking this pathway with PKC-β inhibitors (ruboxistaurin) have been unsuccessful in preventing the progression of diabetic retinopathy [157].

The nonenzymatic protein glycation pathway leads to the activation of advanced glycation end-products (AGEs) through oxidative reactions, and these AGEs cause irreversible chemical changes in proteins [158]. The AGEs may harm the cell in three ways. Firstly, they modify proteins involved in gene transcription [159]. Secondly, they can diffuse out of the cell and modify the extracellular matrix, thus causing cellular dysfunction [160]. Thirdly, the AGEs may alter proteins circulating in the blood, for example albumin [161]. Importantly, Hammes et al. have shown that by inhibiting AGEs in experimental animals, it is possible to prevent structural changes in diabetic retinopathy [162].

Inhibition of oxidative stress generated by the mitochondria abolishes all four pathways; the overproduction of superoxide by the mitochondrial electron-transport chain may be the common activator of the cascades, eventually leading to vascular complications [13] (Figure 1).

2.3.3 Glucose variability

Patients with diabetes show substantial fluctuations in their blood glucose profile throughout the day. In spite of this, very few in vivo studies have investigated the role of glucose variability as a risk factor for diabetic complications. There are, however, in vitro studies suggesting that glucose variability activates cellular changes through the pathways shown in Figure 2 [163] [164]. Furthermore, the clinical difficulties in controlling the HbA_{1c} raise an important question: Whether it would it be better to target glucose variability rather than HbA_{1c} in order to prevent complications.

An Australian study found a correlation between glucose variability and short-term mortality in critically ill non-diabetic patients [9]. These results are consistent with the fact that postprandial hyperglycaemia is a risk factor for cardiovascular disease in type 2 diabetes [7] [165].

In contrast, Kilpatrick et al. showed very recently in patients with type 1 diabetes that glucose variability influences neither the risk for microvascular nor for macrovascular complications [10] [11]. However, daily glucose fluctuations are associated with an activation of oxidative stress in patients with type 2 but not with type 1 diabetes [166] [167]. In a recent study by Ceriello et al. oscillating glucose clearly altered endothelial function and oxidative stress both in patients with type 2 diabetes and in

non-diabetic control subjects [168]. No study has yet shown, in patients with type 1 diabetes, a correlation between glucose variability and long-term or short-term complications.

2.4 Intermediate markers of macrovascular disease

Early detection of diabetic complications is essential in order to start protective treatment as early as possible. A number of intermediate or surrogate markers for vascular complications may therefore be useful for detection in patients with diabetes who show no clinical signs of complications. Arterial stiffness, hypertension, and a prolonged QT time served as intermediate markers to measure vascular complications in the present studies.

2.4.1 Arterial stiffness

2.4.1.1 Definition

The term arterial stiffness indicates that the capability of the arteries to expand and contract during the cardiac cycle is reduced.

2.4.1.2 Molecular mechanisms

Two extracellular proteins, collagen and elastin, are the main determinants of the physical properties of the arteries [169]. A balance between these two proteins is regulated by specific enzymes: metalloproteases. Hypertension leads to increased collagen production and eventually to stiffer arteries [170]. Elastin, on the other hand, provides the arteries with some of their elastic properties. Metalloproteases destroy elastin molecules and thereby increase arterial stiffness [171]. AGEs have also been shown to contribute to arterial stiffening by their formation of irreversible compounds through non-enzymatic reactions between the proteins, collagen and elastin, and glucose [172].

2.4.1.3 Inflammation

A link between stiff arteries and chronic inflammation was recently shown through associations between arterial stiffness and CRP, TNF-α, and Il-6 concentrations in non-diabetic individuals [173] [174] [175]. Stiffness of the large arteries was also associated with CRP in healthy individuals [176] [177]. These results are in line with the fact that both CRP, TNF-α, and Il-6, as well as arterial stiffness, are risk factors for diabetic macrovascular disease [85] [86].

2.4.1.4 Endothelial dysfunction

Arterial stiffness is closely linked to endothelial function and smooth muscle tone in the arteries [178]. The endothelium consists of a single cell layer that separates the blood from the other tissue of the arteries [179], and is an important endocrine organ with autocrine, paracrine, and endocrine functions. The functional role of the endothelial cells differs according to the location of the arteries. In the large arteries, the endothelium is important for inflammatory processes and lipid metabolism.

In the resistance arteries, the endothelium regulates blood flow and blood pressure. This regulation is partially mediated by endothelium-derived vasoactive substances, including prostacyclins, vascular endothelial growth factors, interleukins, nitric oxide (NO), endothelin, ACE, and the von Willebrand factor.

In the capillary bed, the endothelial cells transport nutrients and hormones such as glucose, fat, and insulin [180].

Endothelial dysfunction is characterized by the loss of endothelium-dependent vasodilatation, partly through decreased NO production, and results in increased arterial stiffness [181]. This is considered to represent an early phase in the pathogenesis of cardiovascular disease [182]. Pulse wave analysis can measure endothelial function indirectly [183].

2.4.1.5 Oxidative stress

Studies that investigate markers of oxidative stress and arterial stiffness are few, although it is well known that oxidative stress is a key event in the pathogenesis of diabetic complications [13]. Kampus et al. demonstrated associations between arterial stiffness and oxidized low-density lipoprotein (OxLDL), a marker of oxidative stress, in non-diabetic subjects [184]. Matsuo et al. reported that both arterial stiffness (pulse wave velocity), and oxidative stress (malondialdehyde LDL), were significantly decreased after four weeks of treatment with statins in non-diabetic patients with hypercholesterolemia [185]. Further studies are needed to clarify whether and how oxidative stress may be linked to arterial stiffness in patients with type 1 diabetes.

2.4.1.6 Arterial stiffness measured by applanation tonometry

Arterial stiffness can be measured by several methods: ultrasonography, magnetic resonance imaging, calculation of the ambulatory arterial stiffness index, and pulse contour analysis. Applanation tonometry is a widely used non-invasive method. Pulse wave analysis (PWA) is used to estimate systemic arterial stiffness by analyzing peripheral arterial pressure waveforms. Applanation tonometry can also be used to measure pulse wave velocity (PWV) that reflects the elasticity of both large (aortic) and intermediate (brachial) sized arteries.

Arterial stiffness is measured by PWA; it is associated with coronary artery disease [186] and is a risk factor for cardiovascular disease in patients with established coronary artery disease [187]. It is also a predictor of cardiovascular disease in patients with end-stage renal disease [188] and is consequently a useful surrogate marker for macrovascular disease.

PWV is an independent predictor of cardiovascular disease both in patients with hypertension and in ones with end-stage renal disease [189] [190]. It is, moreover, an independent predictor of mortality in patients with diabetes as well as in the elderly and even in the general population [191] [192] [193], and can therefore serve as an intermediate marker for macrovascular disease.

2.4.1.7 Risk factors for arterial stiffening

The most powerful risk factor for increased arterial stiffness is age [194]. Another important independent risk factor as shown in a number of studies is hypertension [195] [196]. Of note, increased stiffness of the aorta is a risk factor for progression from normal blood pressure to hypertension in the general population [197], in which hypercholesterolemia, as well, correlates with increased arterial stiffness [198]. Data also show that patients with type 1 diabetes have stiffer arteries than do non-diabetic subjects [199] [200] [201] [202] [203]. Regular physical activity protects from arterial stiffening in non-diabetic subjects according to Tanaka et al [204]. Acute stiffening of the arteries during smoking occurs both in smokers and in non-smokers [205] [206]. Not only active but also passive smoking leads to increased arterial stiffness over time [207] [208]. Moreover, the metabolic syndrome [209] (characterized by a clustering of independent cardiovascular risk factors such as central obesity, hypertension, impaired glucose regulation and dyslipidaemia) correlate with arterial stiffening in the general population [210].

2.4.1.8 Treatments affecting arterial stiffness

A wide range of pharmacological and non-pharmacological agents have been used to reduce arterial stiffness [211]. Pharmacological treatment include antihypertensive drugs, hypolipidemic agents, and antidiabetic agents [212]. The Conduit Artery Function Evaluation (CAFE) study showed an important difference in augmenatation index (Aix) and central blood pressure in hypertensive diabetic and non-diabetic patients treated with β-blockers compared with those taking calcium antagonists [213]. β-blockers did not reduce AIx and central BP, but calcium antagonists did. Speculatively this was due to the inability of β-blockers to reduce the magnitude of the reflection wave [214]. This finding is in line with those from the Losartan Intervention For Endpoint Reduction (LIFE) study demonstrating that ACE inhibitors are more effective than β-blockers in reducing left ventricular hypertrophy and its consequences [215]. In addition, preliminary data show beneficial effects of the so-called AGE-breakers on arterial stiffness, a finding that may be of importance especially for patients with diabetes [216].

Non-pharmacological treatments include exercise, weight loss, a low-salt diet, moderate alcohol consumption, and hormone replacement therapy, as well as alternative treatment options such as garlic powder, dark chocolate, and fish oil [217].

2.4.2 Hypertension

Both epidemiological studies and clinical trials have shown that hypertension is an independent risk factor for micro- and macrovascular disease in diabetes. In the general population, a higher risk for cardiovascular disease begins already at blood pressure values as low as >115/75 mmHg [218]. The Hypertension Optimal Treatment (HOT) trial showed that patients with diabetes should maintain a diastolic blood pressure below 80 mmHg [219]. The current guidelines set their target at <130/80 mmHg [220]. Multiple studies have shown in patients with diabetes, that antihypertensive agents protect from microvascular and macrovascular disease [221]. However, the consensus stands that ACE inhibitors (or ARBs) are the drugs of choice in the management of blood pressure in diabetes with or without diabetic nephropathy [222].

2.4.2.1 Pulse pressure

Pulse pressure (PP) is defined as the difference between the systolic (SBP) and the diastolic (DBP) blood pressure, and starts to increase after the age of 55 to 60 years due to a stiffening of the arteries as part of normal ageing [223]. It is a predictor of cardiovascular disease in the general population [224], and in elderly people it has even stronger predictive value than systolic or diastolic pressure alone [225]. The PP has been shown to be associated with the inflammatory markers Il-6 and CRP in healthy men [226 227]. Interestingly, patients with type 1 diabetes show an accelerated ageing of the arteries, a phenomenon that in part may explain their enhanced risk for cardiovascular disease [228]. In the Finnish Diabetic Nephropathy (FinnDiane) Study, the finding was evident both in females and males. In fact, the PP already started to increase 15 to 20 years earlier in patients with type 1 diabetes than in non-diabetic controls.

2.4.3 Prolonged QT interval

QT time on the electrocardiogram (ECG) reflects the total duration of ventricular myocardial depolarisation and repolarisation. The heart-rate corrected QT time (QTc) is shown to predict cardiovascular mortality in healthy subjects as well as in patients with types 1 and 2 diabetes [12 229]. Hence, in these patients, the QTc interval can be serve as an intermediate non-invasive marker of cardiovascular risk.

A few risk factors for QTc prolongation have been identified in the general population, including high blood pressure, female sex, genetic susceptibility, and ischaemic heart disease [230 231 232]. In the EURODIAB prospective complications study, female sex, HbA_{1c}, and systolic blood pressure were

confirmed as risk factors for a prolonged QTc in patients with type 1 diabetes. Clearly, physical activity and a lower BMI play a protective role [233]. QTc time is also associated with diabetic nephropathy and autonomic neuropathy in patients with type 1 diabetes [232].

2.4.3.1 Short-term hyperglycaemia and QT time

Hypoglycaemia is associated with a prolonged QTc interval [234]. It is possible that risk of sudden death, the so-called ”dead in bed” syndrome [235] seen in patients with type 1 diabetes is due to their prolonged QTc time. Marfella et al. in turn reported that acute hyperglycaemia prolongs QT time also in non-diabetic subjects, with similar results in rats [236] [237]. Di Filippo et al. reported that reversal of QT-interval prolongation during acute hyperglycaemia caused by either a specific antioxidant or a endothelin-1 receptor antagonist occurred in rats, suggesting that oxidative stress and endothelial dysfunction are key events in the process [238] [239]. Hence, a link between acute hyperglycaemia and the QTc interval may be oxidative stress, which in turn activates a sympathetic response. Whether chronic hyperglycaemia acts in the same way is still unclear.

2.5 Pre-eclampsia

Pre-eclampsia, a serious disorder that complicates 3 to 5% of all pregnancies [240] [241], is characterized by hypertension, proteinuria, and endothelial dysfunction appearing during the second half of pregnancy [242]. The incidence of pre-eclampsia in patients with type 1 diabetes is higher than in pregnant nondiabetic women, generally exceeding 10% [243]. Pregnancy-induced hypertension (PIH) is also twice as common in patients with type 1 diabetes as in non-diabetic individuals [15].

The pathogenesis of pre-eclampsia is not fully understood, but according to one hypothesis, insufficient invasion of the placental cytotrophoblasts into the uterine spiral arteries causes placental ischaemia, which in turn causes a release of yet-unknown vasoactive factors. These substances damage the maternal endothelium and cause widespread impairment of endothelial function [244].

In patients with type 1 diabetes, the most important risk factors for pre-eclampsia are prepregnancy microalbuminuria, or proteinuria [245] [246] [247]. Other predictive factors are retinopathy, poor glycaemic control, nulliparity, and long duration of diabetes [248]. Although the clinical manifestations of pre-eclampsia disappear after delivery, endothelial dysfunction seems to persist even years after pre-eclamptic pregnancy [249].

Importantly, a history of pre-eclampsia elevates the risk for cardiovascular morbidity and mortality in the general population, but only a few studies have focused on the effects of pre-eclampsia on the microvascular complications in type 1 diabetes [250] [251] [252] [253]. Pregnancy itself does not seem to be a major cause of diabetic microvascular complications [254]. Lovestam-Adrian et al. showed that pre-

eclampsia on the other hand aggravates diabetic retinopathy, but patients in that study were followed for only 6 months after delivery [255]. A small retrospective study showed that proteinuria but not preeclampsia is a risk factor for nephropathy in women with type 1 diabetes [256]. Whether pre-eclampsia leads to diabetic nephropathy is thus still unclear.

3 Aims of the study

The aims of the present studies were to learn:

I. Whether acute hyperglycaemia influences arterial stiffness in patients with type 1 diabetes.

II. Whether daily blood glucose fluctuations influence central and peripheral blood pressure as well as arterial stiffness.

III. Whether acute hyperglycaemia disturbs myocardial repolarization in patients with type 1 diabetes.

IV. Whether inflammatory markers respond to acute hyperglycaemia.

V. Whether pre-eclampsia and pregnancy-induced hypertension predict diabetic nephropathy in patients with type 1 diabetes.

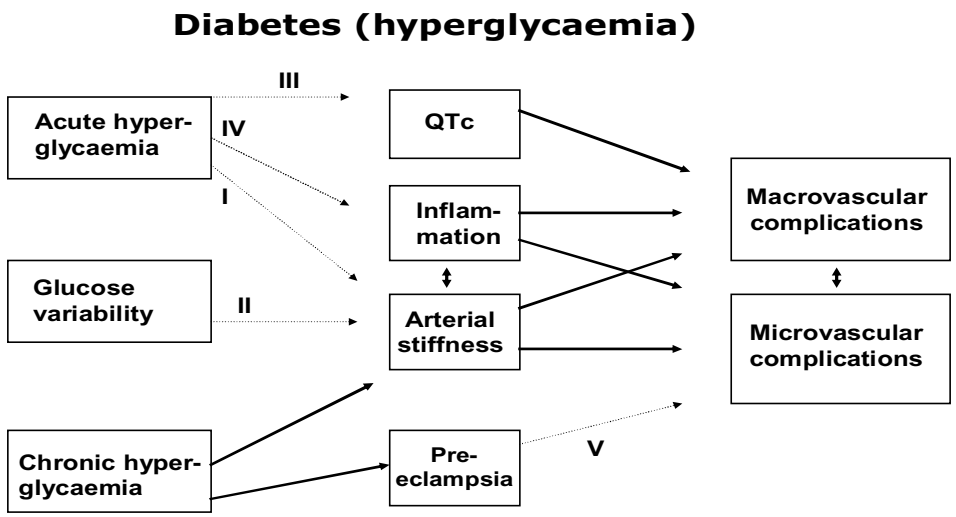


Figure 2. Glycaemia, intermediate markers, and diabetic complications. Roman numerals refer to the five (V) studies.

4 Subjects and study design

4.1 Ethical aspects

All studies were approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa. All subjects gave their written informed consent prior to participation.

4.2 Young patients with type 1 diabetes (I-IV)

The clinical characteristics of all patients are shown in Table 1. Twenty-two male patients with type 1 diabetes were recruited from the FinnDiane study. All these patients participated in Studies I to V. The FinnDiane is a nationwide multicenter study exploring clinical, environmental and genetic risk factors for type 1 diabetes and its complications. All subjects were between 18 and 40 years of age. Exclusion criteria were smoking, hypertension, arrhythmias, diabetic complications, any medical treatment (except insulin), and acute infections. Incipient or overt diabetic nephropathy was ruled out by review of all available urine collections for AER prior to the study visit. Only patients who fulfilled the criteria of a normal AER (<20 μg/min or <30 mg/24h) in two of three overnight or 24 h urine collections were selected. Absence of diabetic retinopathy was verified from their medical files. ECG was recorded from all study subjects, and all recordings were normal. The patients attended a thorough clinical investigation including blood samples. Their blood glucose concentration was monitored for 72 h, after which they underwent at 2 h hyperglycaemic clamp.

4.3 Healthy Volunteers (I-IV)

Table 1 shows the clinical characteristics of the healthy volunteers. Thirteen healthy males served as control group. They were age-matched non-smoking medical students. After a clinical investigation similar to the one performed on patients with type 1 diabetes, a 2 h hyperglycaemic clamp was performed. Normal ECGs were recorded for all the healthy volunteers.

Table 1. Descriptive characteristics of patients and healthy volunteers (Studies I-IV).

	Patients with type 1 diabetes	Healthy volunteers
Age (years)	25.9 ± 5.6	25.4 ± 1.4
Height (cm)	179 ± 6	181 ± 5
BMI (kg/m^2)	23.5 ± 3.4	24.0 ± 1.7
HbA_{1c} (%)	7.4 ± 0.9[a]	5.2 ± 0.3
SBP (mmHg)	123 (116-139)	126 (117-136)
DBP (mmHg)	73 (67-78)	72 (67-78)
Serum cholesterol (mmol/l)	4.3 ± 1.0	4.3 ± 0.7
Serum LDL-cholesterol (mmol/l)	2.2 ± 1.0	2.2 ± 0.7
Serum HDL-cholesterol (mmol/l)	1.7 ± 0.4	1.7 ± 0.4
Serum triglycerides (mmol/l)	0.7 (0.6-1.2)	0.6 (0.5-1.0)
Serum creatinine (µmol/l)	72.6 ± 12.9[b]	83 ± 12.0
Albumin excretion rate (mg/24h)	2.0 (0.0-13.2)	0.0 (0.0-4.0)
Duration of diabetes (years)	9.5 ± 4.4	-

Data are presented as mean ± SD or median with interquartile range. [a] P<0.001, and [b] P<0.01 for change in parameters. BMI = body mass index, DBP = diastolic blood pressure, SBP = systolic blood pressure.

4.4 Women with type 1 diabetes followed during their pregnancy (V)

The clinical characteristics of the patients during pregnancy and at follow-up time are shown in Table 2. The patients (n=429) included at baseline were women with type 1 diabetes who were followed throughout their pregnancy and delivery at the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital during the period 1988 to 1996. In the greater Helsinki area with its population of 1.5 million inhabitants, this is the only center responsible for the obstetric care of women with type 1 diabetes. Out of the 429 baseline patients, 366 had one, 46 had two, and 17 had more than two childbirths (total number of deliveries 590). Some patients could not be tracked; seven had died before the present study, but follow-up data could be retrieved for all seven. The number of patients invited to the follow-up was 396, of whom 196 accepted the invitation.

Table 2. Characteristics of patients (Study V) during pregnancy and at follow-up.

	Normotensive pregnancy (N=105)	Pre-eclampsia (N=43)	Pregnancy-induced hypertension (N=32)
Index pregnancy			
Age (years)	31.1 ±5.2	28.3 ±4.0[a]	28.6 ±5.2[b]
BMI (kg/m^2)	22.5 ±2.8	22.9 ±2.1	23.8 ±3.2[b]
Gestational age at delivery (weeks)	37.5 ±1.4	36.3 ±1.7[a]	37.1 ±1.6
Birth weight Z-score (Sd)	1.2 ±1.9	1.3 ±1.9	1.7 ±1.6
Birth weight (g)	3725 ±811	3433 ±852	3811 ±621
HbA_{1c} prepregnancy (%)	7.5 ±1.1	7.7 ±0.9	7.3 ±0.7
HbA_{1c} I trimester (%)	7.5 ±1.3	8.1 ±1.2	7.0 ±1.6
HbA_{1c} II trimester (%)	7.0 ±1.1	7.3 ±0.9	7.1 ±1.3
HbA_{1c} III trimester (%)	7.2 ±1.2	7.5 ±1.2	7.4 ±1.4
Nulliparity (%)	55.2	81.4[b]	75.0
Follow-up			
Age (years)	41.7 ±6.6	37.9 ±5.9[b]	39.5 ±5.6
BMI (kg/m^2)	24.9 ±4.3	24.6 ±3.3	25.8 ±4.2
Duration of diabetes (years)	24.1 ±8.6	26.8 ±7.5	24.4 ±9.6
SBP (mmHg)	128 ±15	133 ±14	131 ±16
DBP (mmHg)	76 ±10	80 ±8[b]	79 ±8
Serum Total cholesterol (mmol/l)	4.7 ±0.8	4.7 ±0.8	4.7 ±0.7
Serum HDL-cholesterol (mmo/l)	2.0 ±4.7	1.8 ±0.5[b]	1.9 ±0.4
Serum LDL-cholesterol (mmol/l)	2.4 ±0.6	2.5 ±0.8	2.4 ±0.6
Serum Triglycerides (mmol/l)	0.7 [0.3-1.6]	0.9 [0.4-3.1] [b]	0.9 [0.3-2.1] [b]
HbA_{1c} (%)	8.6 ±1.5	8.8 ±1,3	8.7 ±1.6
Serum Creatinine (µmol/l)	74.7 ± 16.0	93.3 ± 55.5[b]	74.5 ± 12.0
AER (mg/24 h)	16.8 [0.1-116]	43.6 [2-293]	9.9 [4.4-18.0]
Smokers (%)	21.7	15.4	32.3
Antihypertensive treatment (%)	9.8	50.0[a]	41.9[a]
Diabetic nephropathy (%)[c]	8.9	41.9[a]	10.3
Coronary heart disease (%)	2.2	12.2[b d]	3.2
Myocardial infarction (%)	0	7.3[b d]	3.2

Data are means ± SD or median with interquartile range. [a]*P*<0.001 and [b]*P*<0.05 vs Normotensive pregnancy. Patients with prepregnancy hypertension were excluded from the analysis (N=23). [c]White F excluded, [d]Fisher's exact test used. SBP = systolic blood pressure, DBP = diastolic blood pressure, AER = albumin excretion rate, BMI = body mass index.

5 Methods

5.1 Definitions

5.1.1 Diabetes

Type 1 diabetes was defined as a diagnosis of type 1 diabetes with age at onset less than 40 years, and insulin therapy initiated within one year (I-IV). In Study V, type 1 diabetes was defined in the same manner despite the fact that age at onset was less than 35.

5.1.2 Hypertension

Essential hypertension was defined as a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or the use of antihypertensive medication in the patients with type 1 diabetes and control subjects (I-V).

5.1.3 Pre-eclampsia and pregnancy-induced hypertension (V)

Pre-eclampsia was defined as elevated blood pressure as described above accompanied by proteinuria after 20 weeks of pregnancy. Pregnancy-induced hypertension was defined as elevated blood pressure in the absence of proteinuria[257 258]. Patients with hypertension before 20 weeks of pregnancy were excluded from all analyses, and patients with proteinuria during the first half of pregnancy (White´s class F) were excluded from the analyses involving nephropathy.

5.2 Pulse wave analysis and velocity (I, II, IV)

5.2.1 Pulse wave analysis

As the left ventricle of the heart contracts, it creates a forward pressure wave that travels to the periphery throughout the arterial tree. As the pressure wave reaches the branching points of the arteries, high-resistance arterioles and regions of increased arterial stiffness, it reflects backwards, towards the heart [259]. The reflected wave results in an arterial waveform that varies throughout the arterial tree and can be measured as the pulse wave analysis. The reflected wave arrives back to the aorta in elastic arteries during diastole, augmenting diastolic pressure and improving coronary perfusion. As arterial stiffness increases, the reflected wave returns to the heart at an earlier phase of

the cardiac cycle, augmenting, instead of the diastolic pressure, the systolic pressure, and causing a reduction in coronary perfusion and an increase in cardiac oxygen consumption. This may, over time, result in left ventricle hypertrophy and manifest heart disease.

Pulse wave analysis (PWA) was recorded by an applanation tonometer (SphygmoCor, Atcor Medical, Sydney, Australia). This method estimates arterial stiffness by analyzing peripheral arterial pressure waveforms with a high-fidelity micromanometer (SPC-301; Millar Instruments, Houston, TX, USA) [260]. The pressure waveforms were recorded from the radial artery of the right arm with the wrist slightly extended and supported on a pillow. The data were collected directly into a desktop computer and processed with the SphygmoCor software using a validated generalized transfer function was based on a comparison with intra-arterial pressures in patients having surgery. This function was then applied to generate the corresponding central aortic waveform [261 262]. The augmentation can be calculated as the difference between the second (caused by wave reflection) and the first systolic peak (caused by ventricular ejection) (Figure 3). The average of three consecutive readings, each consisting of at least 20 sequentially recorded waveforms, served for the analyses. The augmentation index (AIx), a generally used variable to estimate arterial stiffness, was then calculated by the software as the quota of the augmentation and the central pulse pressure. Reproducibility of this method is in accordance with that reported by other investigators [263 266]. When the heart rate is high, the pulse wave reflection returns to the aorta at an earlier phase of the cardiac cycle. Thus, heart rate and AIx are in an inverse association, and AIx must be adjusted for heart rate to avoid inaccurate results [264 265]. All measurements were subjected to internal quality control by the software.

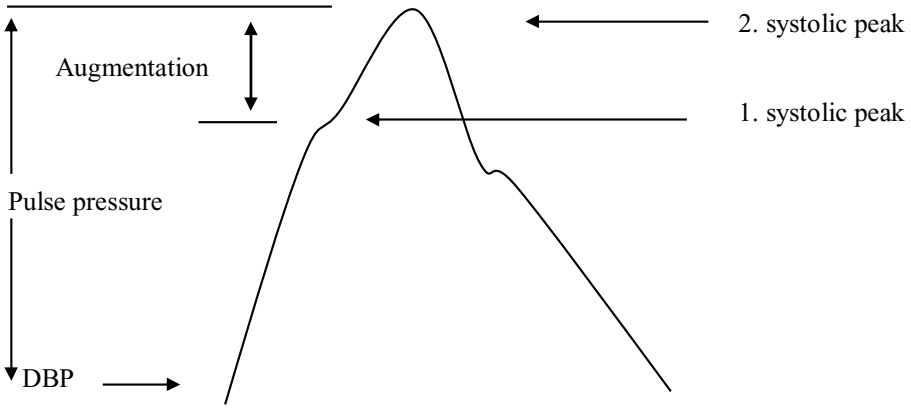


Figure 3. Pulse wave of aorta measured by applanation tonometer. DBP = diastolic blood pressure.

5.2.2 Pulse wave velocity

Pulse wave velocity (PWV) is the speed at which the forward pressure wave is transmitted from the aorta through the vascular tree. In order to measure arterial stiffness in large (aortic) and intermediate (brachial) -sized arteries, the carotid-femoral (aortic) and carotid-radial (brachial) pulse wave velocity pressure waveforms are recorded sequentially at both the carotid, femoral, and radial arteries. This is performed with the SphygmoCor device by sequentially recording ECG-gated carotid and femoral artery waveforms with a high-fidelity micromanometer for 30 seconds. The difference between carotid-to-femoral and carotid-to-radial path length was estimated from the distance from the sternal notch to the femoral and carotid palpable pulse. Assessment of pulse wave analysis and pulse wave velocity represent a relatively simple technique that has been widely applied and found to be robust and reproducible. The software calculated the pulse wave velocity as previously described [266].

5.3 Blood pressure (I-V)

Aortic (central) blood pressure was also determined by applanation tonometry (I, II, IV). Brachial blood pressure was measured in duplicate from the left arm by a validated oscillometric sphygmomanometer (Omron Corp, Bannockburn, IL, USA) (I-V). The mean of the recordings were calculated and used for the analyses.

5.4 72 h continuous glucose monitoring (II)

A continuous glucose monitoring system (CGMS) was used to study the 72 h interstitial fluid glucose profile. The CGMS system (Medtronic MiniMed, Sylmar, CA, USA) is accepted for use as a Holter-type monitor and has been validated and accepted by the U.S. Food and Drug Administration [267 268 269]. The patients were told to modify their usual daily behaviour as little as possible during the test. The system was well tolerated by all patients. The CGMS is a minimally invasive glucose sensing system that is inserted into the abdominal subcutaneous fat to record interstitial fluid glucose concentrations between 2.2 and 22.0 mmol/l. The glucose monitor recorded interstitial fluid glucose concentrations in each patient every fifth minute for 72 h and needed calibration by four blood glucose values daily obtained by finger sticks. After 3 days, the data were downloaded via the Com-Station by the MiniMed Solutions Software version 2.0b (Medtronic MiniMed), and the 24 h glucose profile obtained for each of the 3 days was analyzed. As an index of hyperglycaemic episodes, the area under the curve for values exceeding 10 mmol/l (AUC+10) was calculated from each 24 h glucose profile falling within the range (2.2-22.0 mmol/l). The area under the curve below 5 mmol/l (AUC-5) was used for low glucose excursions, meaning

hypoglycaemia (Figure 4) [270]. Moreover, the glucose curves were manually inspected to verify that the sensors had functioned as intended. The mean amplitude of glycaemic excursions (MAGE) was calculated to describe glucose fluctuations during the day [271]. MAGE detects major swings of glycaemia but excludes minor ones. Calculation of MAGE was performed by measurement of the arithmetic mean of the differences between consecutive peaks and nadirs. The analyses were performed with in-house scripts in the Mathlab programming environment (MathWorks Inc, Natick, MA, USA).

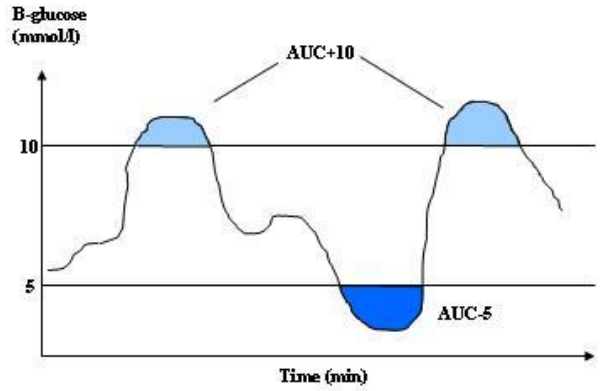


Figure 4. Schematic figure of hyperglycaemic episodes (AUC+10) and low glycaemic excursions (AUC-5) by the continuous glucose monitoring system (CGMS).

5.5 2 h hyperglycaemic clamp (I-IV)

At the end of the 72 h glucose monitoring, the subjects visited the research centre. All subjects had been instructed to fast overnight and, to refrain from smoking and from drinking alcoholic beverages or coffee from the night before the study. They were instructed to take half of their long-acting insulin dose (full dose in the case of glarginine insulin) in the morning. They rested for more than 30 minutes prior to the clamp. Intravenous lines were inserted into a large antecubital vein of the right arm for infusions and into a dorsal vein of the left arm for blood sampling.

During the hyperglycaemic glucose clamp, blood glucose concentrations were acutely raised with a bolus injection of 0.25 g/kg glucose (50% solution) followed by a variable 20% glucose infusion to achieve steady-state plasma glucose levels of about 15 mmol/l for 120 minutes. Blood samples were drawn every 10 minutes from retrograde cannulas (arterialized venous blood) to measure and adjust the blood glucose levels (Beckman Instruments Inc, Fullerton, CA, USA).

The healthy volunteers were examined in the same manner except that prior to the glucose bolus they received a 25 µg bolus followed by a 0.5 µg/min infusion of a somatostatin analogue (Sandostatin®, Novartis, Finland) to inhibit their endogenous insulin production. Somatostatin was infused throughout the clamp through a third cannula inserted in the left arm (antecubital vein).

Measurements of blood pressure, arterial stiffness, and ECG were made before (at normoglycaemia) and at 0, 60, and 120 minutes of hyperglycaemia (Figure 5). Blood samples were drawn at baseline and at the end of the 2 h clamp. Additionally, in the healthy volunteers, an additional measurement was made after the somatostatin analogue infusion but before the glucose bolus. All measurements were performed by a single operator.

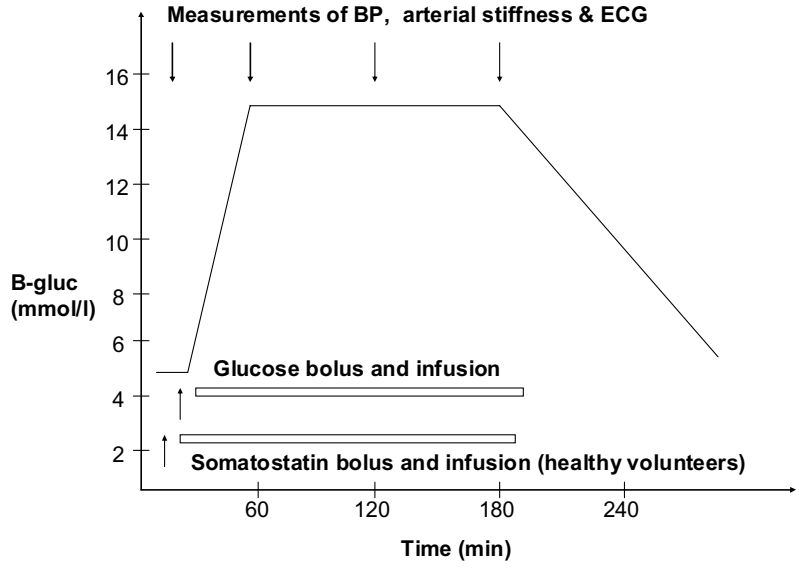


Figure 5. Schematic figure of the 2 h hyperglycaemic clamp.

5.6 Measurement of QT interval and dispersion (III)

The ECGs were recorded in the supine position from standard 12-lead bands at 50 mm/s, the amplitude calibration being 10 mm/mV for all readings. Eight of the 12 leads were evaluated. In each lead, three consecutive complexes were read. No recordings of extrasystoles or subsequent beats were analyzed. The analysis was made according to the Minnesota code [272]. All tracings were analysed manually by the same physician (DG) twice in random order, and the physician was blinded to all clinical data from individual patients. The QT interval length was measured from the onset of the QRS complex to the end of the T wave. In the presence of U waves, the end of the QT interval was set at the nadir of the curve between the T and the U wave. Maximal QT interval was corrected for each heart rate by the Bazett formula ($QTc_{Bazett} = QT/RR^{1/2}$) [273], and in addition, the formula suggested by Fridericia ($QTc_{Fridericia} = QT/RR^{1/3}$) [274] and the Framingham formula derived by linear regression ($QTc_{Sagie} = QT + 0.154(1-RR)$ [275]. The QT time was also corrected by the nomogram method ($QT_{Nc} =$ QT + correcting number) [276].

QTc dispersion was calculated as an interlead variability of the QTc interval (QTc -dispersion = QTc max - QTc min), reflecting the degree of inhomogeneity of myocardial repolarisation.

5.7 Biochemical analyses (I-V)

Haemoglobin, leukocyte count, HbA_{1c}, lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), serum creatinine, potassium, sodium, calcium, and albumin were determined from fasting blood samples. Haemoglobin, leukocyte count, potassium, sodium, calcium, and albumin were measured by routine methods. HbA_{1c} was analyzed by immunoturbidimetry (normal range 4.0-6.0%). Serum lipids were measured by automated enzymatic methods with a Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland) and serum creatinine by routine enzymatic methods. Serum insulin was measured by immunofluorometry. Urinary albumin excretion rate (AER) was assessed from a 24 h urine collection by immunoturbidimetry.

Measurements of serum interleukin-6 (IL-6) and plasma tumor necrosis factor-α (TNF-α) were done after storage at -20 ºC by immunochemoluminometry and serum-sensitive C-reactive protein (CRP) by photometry all in duplicate. An acute phase inflammatory marker score was calculated as: (C-reactive protein+IL-6+TNF-α)/3 [277]. Serum samples for determination of intracellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1) and eSelectin were stored at -20ºC until assayed. The concentrations were measured in duplicate with commercially available immunosorbent kits (R&D Systems, Minneapolis, MN, USA). The determination of endothelin-1 (ET-1) was also done in duplicate from extracted plasma (stored at -20ºC) (R&D Systems). The samples for determination of serum superoxide dismutase (SOD) were stored at -80ºC according to

manufacturer's instructions, and measured with a commercially available kit (Cayman Chemical, Michigan, MI, USA).

5.8 Assessments during pregnancy and at follow-up (V)

5.8.1 Blood pressure and kidney function during pregnancy

During pregnancy, blood pressure was measured at each visit in the sitting position after a 10-minute rest. Measurements were made with a sphygmomanometer by midwives and nurses, and blood pressure was considered elevated when the DBP was repeatedly ≥90 mmHg or if it increased by a minimum of 15 mmHg during pregnancy. Urinary protein was measured by a dipstick method at every visit. If the dipstick repeatedly showed "+" or a "++", proteinuria was confirmed by a 24 h urine collection. Proteinuria was defined as urinary protein excretion ≥300 mg/24h.

5.8.2 Glycaemic control during pregnancy

During pregnancy, HbA_{1c} was measured monthly by HPLC (Diamat, Bio-Rad Laboratories, Hercules, CA, USA). The normal range was defined as HbA_{1c} between 4.0 and 6.0%. The first HbA_{1c} assessment during pregnancy was carried out during the period between the 7^{th} and the 14^{th} week of gestation. The mid-pregnancy value was obtained between the 20^{th} and the 25^{th} week and the third measurement approximately 2 weeks before delivery. The average HbA_{1c} value of each trimester was used in the analysis.

5.8.3 Medical history and kidney function at follow-up (FinnDiane visit)

Data on medication, cardiovascular status, and diabetic complications were recorded from a standardized questionnaire completed by the patient´s attending physician and thus immediately verified from the medical files. Coronary heart disease was defined as a positive history of myocardial infarction, bypass operation, a diagnostic finding in angiography or positive exercise test.

Classification of renal status was based on the AER in at least two of three urine-collections at follow-up. Patients were defined as normoalbuminuric (n=135) if their AER was persistently <20 µg/min overnight or <30 mg/24h in the 24h urine collection. Microalbuminuria or incipient diabetic nephropathy (n=24) was defined as an AER between ≥20 <200 µg/min or ≥30 <300 mg/24h, whereas macroalbuminuria or established diabetic nephropathy (n=9) was defined as an AER ≥200 µg/min or ≥300 mg/24h. Patients on renal replacement therapy (dialysis or kidney transplantation) were considered to have end-stage renal disease (ESRD) (n=2). Patients with either

microalbuminuria, macroalbuminuria or clinical ESRD were pooled and considered in the analyses to represent diabetic nephropathy.

5.9 Statistical methods

All analyses were performed with SPSS 13.0 (SPSS, Chicago, IL, USA). Power calculations were performed to test the required size of the study population [278]. Results are presented as mean ± SEM or ± SD for normally distributed variables and as median (interquartile range, IQR) for non-normally distributed variables. P-values <0.05 were considered statistically significant.

Differences between the groups for normally distributed variables were tested with ANOVA or Student´s *t*-test and for non-normally distributed variables with appropriate tests. For categorical variables the χ^2 test or Fisher´s exact test was used when appropriate (V). Simple linear regression was used to examine univariate correlations (Pearson's parametrical test, or Spearman's non-parametrical test). More complex correlations were analyzed by means of multivariate regression analysis.

To detect differences in response to hyperglycaemia within and between the groups, a two-way ANOVA for repeated measures was performed followed by a mixed effects model or Bonferroni's test adjusted for age (I,III). In Study IV, values at different time-points during the hyperglycaemic clamp were compared with paired samples tests.

6 Results

6.1 Clinical characteristics (I-IV)

The baseline clinical characteristics were shown in Table 1. As expected, patients with type 1 diabetes had higher HbA_{1c} (7.4 ± 0.9% vs. 5.2 ± 0.3%, P<0.001) than did healthy volunteers. No differences regarding age, BMI, lipid profile, blood pressure or AER were observable between groups.

6.2 Haemodynamic variables in the study groups at baseline (I-IV)

The haemodynamic variables are depicted in Table 3. Patients with type 1 diabetes had stiffer arteries at baseline (normoglycaemia) than did healthy volunteers after correcting for age (AIx; -5 ± 3% vs. -20 ± 5%, P<0.05) (I). Neither brachial, nor aortic PWV differed between groups at baseline. A statistically significant difference appeared between patients with type 1 diabetes and healthy volunteers regarding oxidative stress at baseline. No difference emerged in QTc (Table 3).

Table 3. Haemodynamic parameters in patients with type 1 diabetes and healthy volunteers at baseline.

	Patients with type 1 diabetes (N = 22)	Healthy volunteers (N = 13)
Brachial SBP (mmHg)	123 (116-139)	126 (117-136)
Brachial DBP (mmHg)	73 (67-78)	72 (67-78)
Brachial PWV (m/s)	7.1 ± 1.2	7.4 ± 1.7
AIx (%)	-5 ± 3 [a]	-20 ± 5
Aortic SBP (mmHg)	105 (99-114)	107 (98-117)
Aortic DBP (mmHg)	73 (67-80)	72 (68-78)
Aortic PWV (m/s)	6.5 (5.8-7.4)	6.5 (5.3-7.1)
QTc (ms)	390 ± 6	378 ± 5
QTc dispersion (ms)	45 ± 3	36 ± 4
Heart rate (beats/min)	62 ± 2	58 ± 3

Data are presented as mean ± SD or median with interquartile range. [a] P<0.05 for change in parameters. SBP = systolic blood pressure, DBP = diastolic blood pressure, AIx = augmentation index, PWV = pulse wave velocity. Formulae for QTc_{Bazett} were used.

6.3 Acute hyperglycaemia and haemodynamic variables (I, III, IV)

Blood glucose concentrations during the clamp in patients with type 1 diabetes and non-diabetic control subjects are shown in Table 4 and Figure 6. Serum insulin in patients with type 1 diabetes increased from 0.3 to 0.6 mU/l during the clamp, while insulin secretion was blocked by the somatostatin analogue in the healthy volunteers (3.3 to 7.2 mU/l).

Table 4. Haemodynamic variables in patients with type 1 diabetes (T1D) (N = 22) and healthy volunteers (N = 13) during a hyperglycaemic clamp.

	Normoglycaemia at baseline	Hyperglycaemia		
		0 min	60 min	120 min
T1D				
Blood glucose (mmol/l)	6.7 (6.3-8.2)	17.3 (15.9-19.0)[a]	17.9 (17.1-20.1)[a]	17.9 (16.8-19.5)[a]
Brachial SBP (mmHg)	123 (116-139)	127 (121-136)	128 (118-136)	126 (121-138)
Brachial DBP (mmHg)	73 (67-78)	72 (66-80)	75 (69-79)	71 (68-80)
Brachial PP (mmHg)	53 ± 11	57 ± 13[a]	55 ± 13	55 ± 9
Aortic SBP (mmHg)	105 (99-114)	110 (103-119)	107 (104-119)	107 (104-115)[a]
Aortic DBP (mmHg)	73 (67-80)	73 (68-83)	75 (69-79)	71 (68-84)
Aortic PP (mmHg)	31 (28-38)	35 (32-41)[a]	35 (30-41)[a]	38 (31-40)[a]
Healthy volunteers				
Blood glucose (mmol/l)	5.1 (4.7-5.4)	16.6 (16.1-17.6)[a]	18.3 (17.6-19.3)[a]	16.6 (16.0-18.4)[a]
Brachial SBP (mmHg)	126 (117-136)	125 (118-134)	128 (117-134)	126 (119-131)
Brachial DBP (mmHg)	72 (67-78)	74 (67-81)	71 (62-78)	68 (63-71)
Brachial PP (mmHg)	56 ± 9.3	49 ±10.7	56 ± 7.3	57 ± 7.6
Aortic SBP (mmHg)	107 (98-117)	106 (103-119)	106 (98-118)	105 (100-111)
Aortic DBP (mmHg)	72 (68-78)	75 (68-82)	71 (63-80)	68 (64-73)
Aortic PP (mmHg)	34 (27-38)	34 (30-41)	36 (34-41)[a]	37 (34-43)[a]

Data are presented as mean ± SD or median with interquartile range. [a] $P<0.05$ for change in parameter 0, 60 or 120 min. of hyperglycemia vs. normoglycemia. SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure.

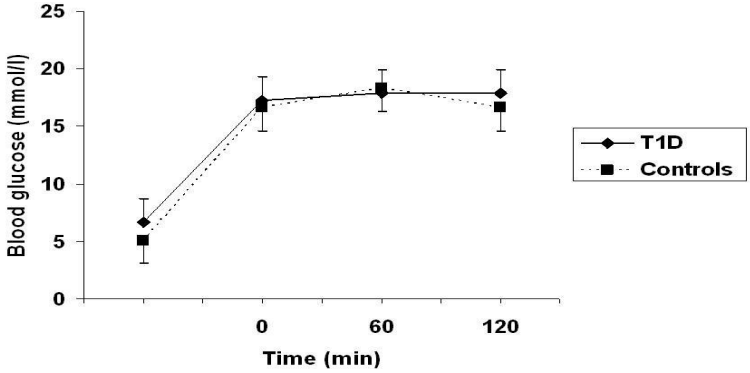
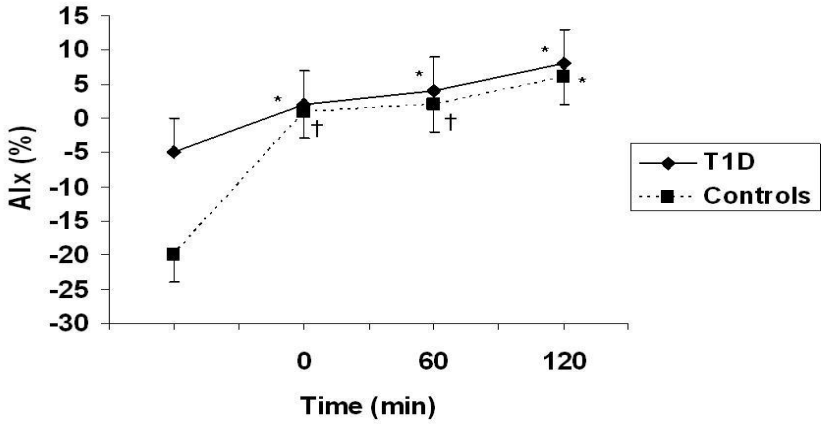


Figure 6. Blood glucose concentration at different time-points in the two study groups. Duration of hyperglycaemia: 0 to 120 min. T1D = Patients with type 1 diabetes, Controls = healthy volunteers. Data are mean±SEM.

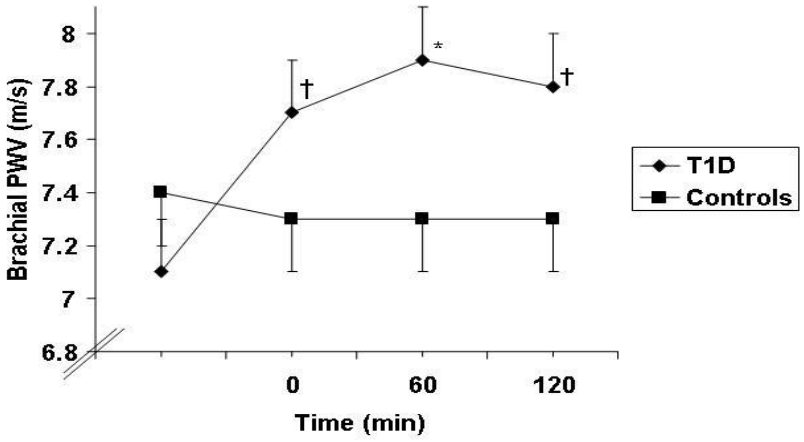
6.3.1 Arterial stiffness (I)

After 120 minutes of hyperglycaemia, AIx increased steeply from -5% (IQR, -20-2) at baseline to 8% (-1-13) ($P<0.001$, Figure 7A) in patients with type 1 diabetes. The same trend was observable in healthy volunteers (-20% (-24-[-9]) vs. 6% (-6-11) , $P<0.001$, Figure 7A). Brachial PWV increased during acute hyperglycaemia compared to normoglycaemia in the patients with type 1 diabetes (7.1 ± 1.2 m/s vs. 8.0 ± 1.0 m/s, $P<0.001$), but not in the healthy volunteers (7.4 ± 1.7 m/s vs. 7,3 ± 1.4 m/s, NS) (Figure 7B). Aortic PWV remained unchanged in both groups (Figure 7C).

A)


15
10
5
0
-5
-10
-15
-20
-25
-30
AIx (%)
0
60
120
Time (min)
T1D
Controls

B)


8
7.8
7.6
7.4
7.2
7
6.8
Brachial PWV (m/s)
0
60
120
Time (min)
T1D
Controls

C)

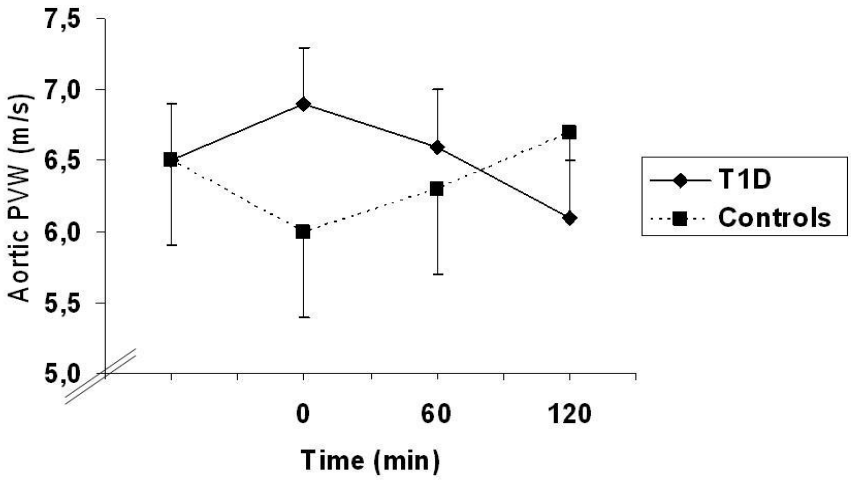


Figure 7. AIx (A), Brachial PWV (B), and Aortic PWV (C) at different time-points in the two study groups. Duration of hyperglycaemia: 0 to 120 min. T1D = Patients with type 1 diabetes, Controls = healthy volunteers. Data are mean±SEM. * $p<0.001$ and † $p<0.05$ for change at 0, 60, and 120 min of hyperglycaemia vs normoglycaemia.

6.3.2 Blood pressure (I)

Neither brachial systolic nor diastolic blood pressure changed during acute hyperglycaemia in either group (Table 4). However, brachial pulse pressure increased in the patients with type 1 diabetes during the clamp (53 ± 11 mmHg vs. 57 ± 9 mmHg, $P<0.05$), although it decreased in healthy volunteers (56 ± 9 mmHg vs. 49 ± 8 mmHg, $P<0.05$). Notably, after 120 minutes of hyperglycaemia, aortic PP increased from 31 (28-38) mmHg at baseline to 38 (31-40) mmHg ($P<0.05$) in patients with type 1 diabetes and from 34 (27-38) mmHg to 37 (34-43) mmHg ($P<0.05$, Figure 8) in healthy volunteers.

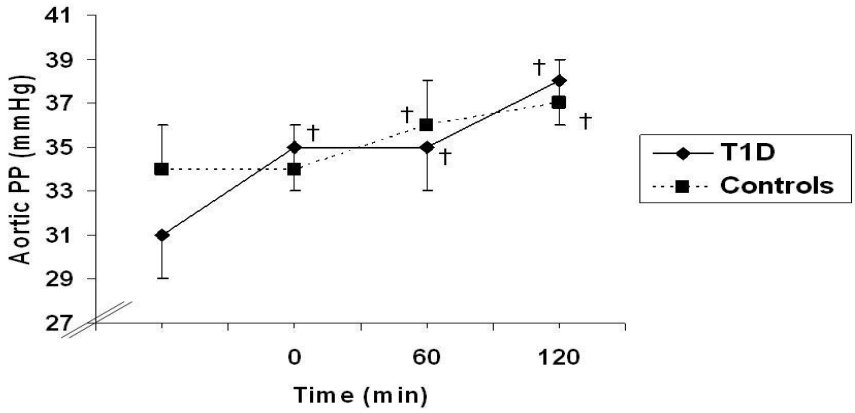


Figure 8. Aortic pulse pressure at different time-points in the two study groups. Duration of hyperglycaemia: 0 to 120 min. T1D = Patients with type 1 diabetes, Controls = healthy volunteers. Data are mean±SEM. † $p<0.05$ for change at 0, 60, and 120 min of hyperglycaemia vs normoglycaemia.

6.3.3 QT time (III)

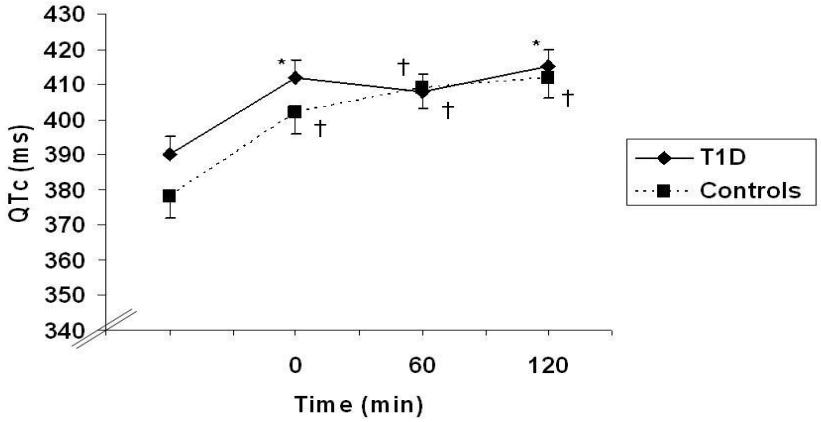
The QTc_{Bazett} increased steeply both in patients with type 1 diabetes (390 ± 6 ms vs. 415 ± 5 ms, $P<0.05$, Figure 9A) and healthy volunteers (378 ± 5 ms vs. 412 ± 8 ms, $P<0.05$, Figure 9A) when the blood glucose was acutely elevated. The same trend was observed for $QTc_{Fridericia}$ and QTc_{Sagie} (Table 5). An elevation in QT dispersion was evident at 60 min of hyperglycaemia in healthy volunteers but not in patients with type 1 diabetes (Figure 9B). The PR interval did not change during acute hyperglycaemia (Table 5). Serum calcium decreased from 2.27 ± 0.02 mmol/l at baseline to 2.17 ± 0.02 mmol/l in patients with type 1 diabetes and from 2.27 ± 0.02 mmol/l to 2.13 ± 0.02 mmol/l in non-diabetic subjects, at 120 min of hyperglycaemia ($P<0.001$). Furthermore, serum potassium changed from 4.8 ± 0.1 mmol/l at baseline to 4.6 ± 0.1 mmol/l at 120 min of hyperglycaemia ($P<0.05$) in patients with type 1 diabetes, but not in healthy volunteers (4.8 ± 0.1 mmol/l to 4.7 ± 0.1 mmol/l, NS).

Table 5. Haemodynamic variables in patients with type 1 diabetes (T1D) (N = 22) and healthy volunteers (N = 13) during a hyperglycaemic clamp.

	Normoglycaemia at baseline	Hyperglycaemia		
		0 min	60 min	120 min
T1D				
Serum sodium (mmol/l)	142 ± 0.6			136 ± 0.6 [a]
Serum potassium (mmol/l)	4.8 ± 0.1			4.6 ± 0.1 [c]
Serum calcium (mmol/l)	2.27 ± 0.02			2.17 ± 0.02 [a]
$QTc_{Fridericia}$ (ms)	385 ± 10	406 ± 11 [b]	413 ± 11 [b]	396 ± 11
QTc_{Sagie} (ms)	387 ± 7	407 ± 6 [a]	410 ± 6 [a]	403 ± 6 [b]
QT_{Nc} (ms)	391 ± 5	412 ± 5 [b]	410 ± 4 [b]	412 ± 4 [b]
Heart rate (beats/min)	62 ± 2	62 ± 2	60 ± 2	65 ± 3
PR time (ms)	158 ± 4	160 ± 4	159 ± 4	161 ± 4
Healthy volunteers				
Serum sodium (mmol/l)	143 ± 0.5			139 ± 0.7 [a]
Serum potassium (mmol/l)	4.8 ± 0.1			4.7 ± 0.1
Serum calcium (mmol/l)	2.27 ± 0.02			2.13 ± 0.02 [a]
$QTc_{Fridericia}$ (ms)	401 ± 17	445 ± 12 [a]	441 ± 16 [b]	426 ±11 [b]
QTc_{Sagie} (ms)	391 ± 9	426 ± 7 [a]	427 ± 11 [a]	420 ± 7 [a]
QT_{Nc} (ms)	382 ± 5	411 ± 5 [b]	416 ± 8 [b]	415 ± 6 [a]
Heart rate (beats/min)	58 ± 3	54 ± 2	56 ± 2	58 ± 2
PR time (ms)	164 ± 5	165 ± 5	168 ± 5	170 ± 4

Data are presented as mean ± SEM or median with interquartile range. [a] P<0.001, [b] P<0.01 and [c] P<0.05 for change in parameter 0, 60 or 120 min. of hyperglycaemia vs. normoglycaemia. HR = heart rate.

A)



B)

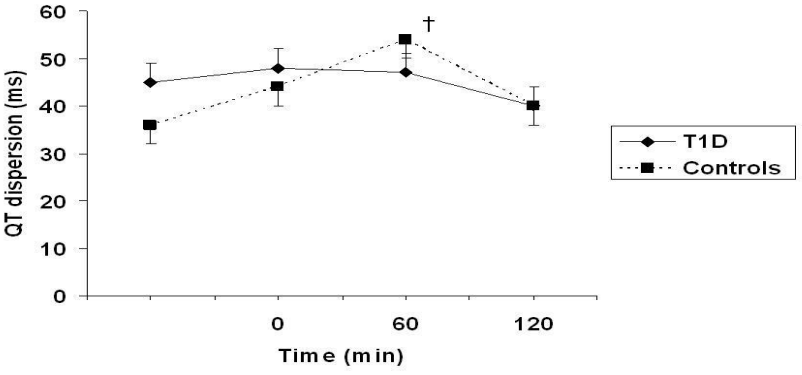


Figure 9. The QTc interval (A) and QTc dispersion (B) at different time-points in the two study groups. Duration of hyperglycaemia: 0 to 120 min. T1D = Patients with type 1 diabetes, Controls = healthy volunteers. Data are mean±SEM. † $p<0.05$ for change at 0, 60, and 120 min of hyperglycaemia vs normoglycaemia. QTc calculated by Bazett's formula.

6.3.4 Inflammation (IV)

Il-6 increased during acute hyperglycaemia both in patients with type 1 diabetes (from 3.1 ± 1.1 to 4.3 ± 1.2 ng/l, P<0.01) and in healthy volunteers (from 2.3 ± 0.3 to 2.9 ± 0.4 ng/l, P<0.01) (Figure 10), but no change in CRP occurred in response to acute hyperglycaemia in either group. TNF-α increased in response to acute hyperglycaemia in patients with type 1 diabetes (3.4 ± 0.2 to 4.6 ± 0.8 ng/l, P<0.05) but not in healthy volunteers (3.9 ± 0.4 to 3.5 ± 0.4 ng/l, NS). The acute phase inflammatory marker score was elevated during acute hyperglycaemia in patients with type 1 diabetes (from 2.7 ± 0.4 to 3.4 ± 0.5, P<0.01) but not in healthy volunteers (from 2.4 ± 0.2 to 2.4 ± 0.3, NS)

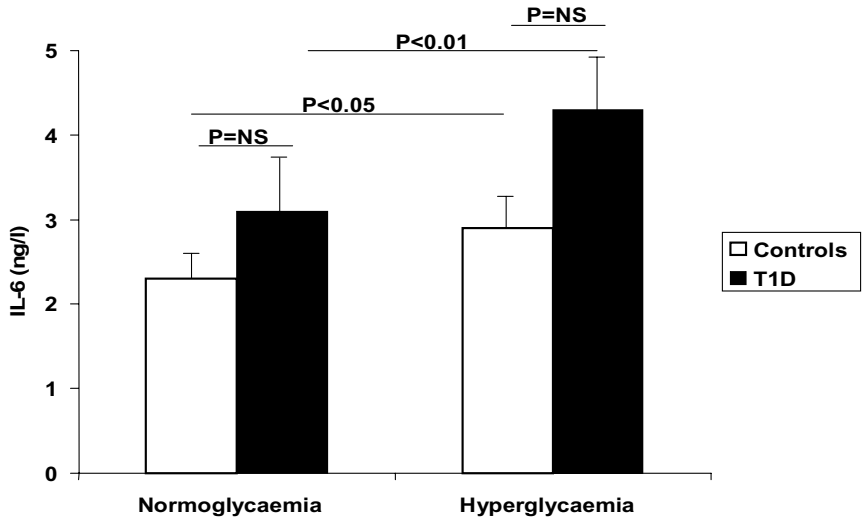


Figure 10. Interleukin-6 (IL-6) during normoglycaemia and hyperglycaemia in both groups. Data are mean ± SEM. T1D = patients with type 1 diabetes, Controls = healthy volunteers.

6.3.5 Endothelial function (IV)

VCAM concentrations remained unchanged in response to acutely elevated glucose concentrations both in patients with type 1 diabetes (498 ± 20 vs. 498 ± 21 ng/ml, NS) and in healthy volunteers (535 ± 40 to 559 ± 39 ng/ml, P=NS). No change was observable in either ICAM or eSelectin in either groups.

6.3.6 Oxidative stress (IV)

Serum SOD levels were higher in patients with type 1 diabetes than in the non-diabetic control subjects at baseline but showed no significant variation during the clamp (0.37 ± 0.03 vs. 0.39 ± 0.03 U/ml, NS) (Figure 11). However, in the healthy volunteers sSOD levels increased from a basal value of 0.29 ± 0.01 to 0.34 ± 0.02 U/ml (P<0.01) during acute hyperglycaemia.

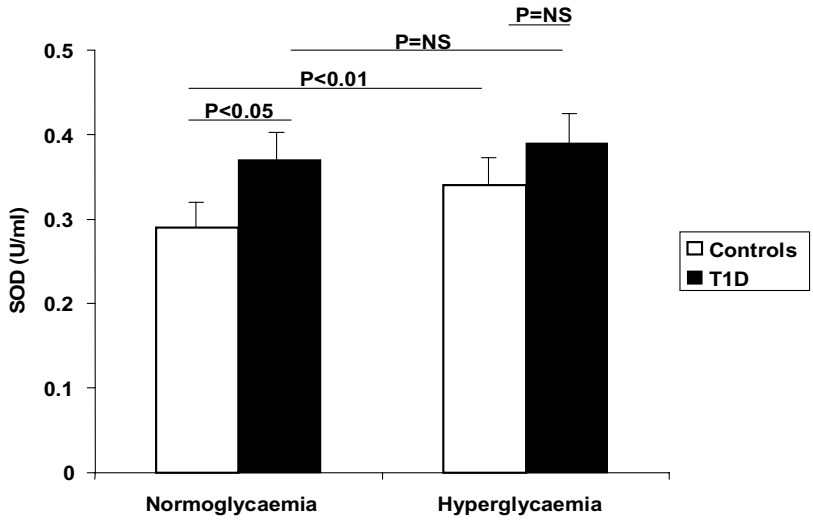


Figure 11. Superoxide dismutase (SOD) during normoglycaemia and hyperglycaemia in both groups. Data are mean ± SEM. T1D = patients with type 1 diabetes, Controls = healthy volunteers.

6.4 Glucose variability and vascular parameters (II)

6.4.1 Glucose variability and haemodynamic variables during normoglycaemia (II)

In the present study, we observed no correlation between MAGE (glucose variability) and any of the measures of arterial stiffness (AIx, brachial or aortic PWV) in univariate regression analysis. A univariate correlation was, however, apparent between HbA_{1c} and AIx at baseline (normoglycaemia).

Mean daily glucose concentrations and aortic PWV correlated positively with each other (Figure 12), but we found no univariate correlations between AUC+10 (postprandial hyperglycaemia) and the haemodynamic variables. A negative correlation was, however, apparent between AUC-5 and aortic PWV at baseline.

An independent association emerged between mean glucose concentrations and aortic PWV (r=0.48, $P<0.01$) after adjustments for BMI, HbA_{1c}, and duration of diabetes. This relationship was independent of age, HR, SBP, HbA_{1c}, and HDL-cholesterol but not of LDL-cholesterol. We further observed independent relationships between AUC-5 and aortic PWV (r=-0.60, $P<0.01$) when adjusted for BMI, HbA_{1c}, and duration of diabetes in multivariate linear regression analysis.

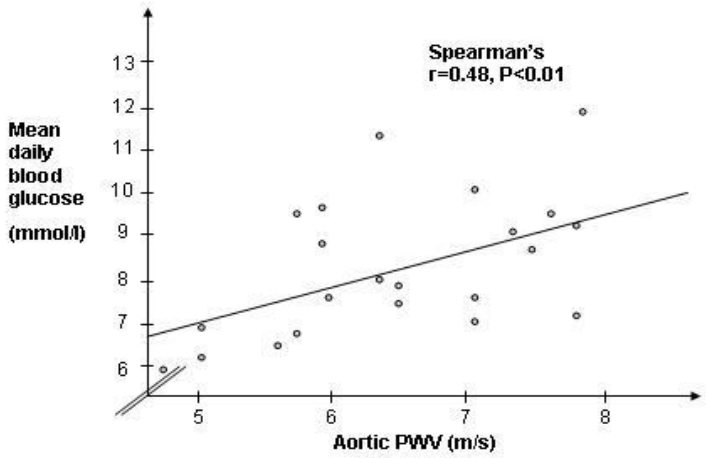


Figure 12. Relationship between mean daily blood glucose concentration and aortic PWV (arterial stiffness).

6.4.2 Glucose variability and haemodynamic variables during acute hyperglycaemia (II)

The changes (Δ) in haemodynamic variables measured between acute hyperglycaemia and baseline values were calculated at both 0 and 120 minutes after the steady state level of hyperglycaemia was reached (Table 6).

None of the measures of glucose control correlated with the change in arterial stiffness (AIx, brachial and aortic PWV) during the clamp. A correlation between MAGE and both Δ aortic SBP and Δ aortic DBP were observed at 0 minutes but not at 120 minutes.

The relationships between MAGE and Δ aortic SBP (r=0.62, $P<0.05$) and between MAGE and Δ aortic DBP (r=0.52, $P<0.01$) were independent of BMI, HbA_{1c}, and duration of diabetes in multivariate regression analysis. Independent relationships also appeared between MAGE and Δ aortic SBP as well as between MAGE and Δ aortic DBP after adjustments for SBP, HbA_{1c}, HDL-cholesterol, and LDL-cholesterol.

No independent relationships appeared between HbA_{1c} and changes in haemodynamic variables during the clamp.

Table 6. Univariate correlation coefficients between a continuous glucose monitoring system and changes (Δ) in central blood pressure during acute hyperglycaemia. The changes (Δ) in aortic BP between acute hyperglycaemia and baseline values were calculated at both 0 and 120 minutes after the steady state level of hyperglycaemia was reached.

	HbA_{1c}	MAGE	Mean daily glucose
At 0 min of hyperglycaemia			
Δ Aortic SBP	0.09 (0.01)	0.45 [a] (0.47 [a])	0.19 (0.40)
Δ Aortic DBP	-0.02 (-0.12)	0.43 [a] (0.57 [a])	0.20 (0.58 [a])
At 120 min of hyperglycaemia			
Δ Aortic SBP	0.36 (0.37)	0.34 (0.21)	0.26 (0.15)
Δ Aortic DBP	0.21 (0.36)	0.16 (0.15)	0.21 (0.39)

[a] P<0.05. The values are univariate correlation coefficients. Correlation coefficients adjusted for HbA_{1c}, brachial systolic blood pressure, and HDL-cholesterol in the linear regression analysis in parenthesis. Changes (Δ) in aortic BP between acute hyperglycaemia and baseline values were calculated at both 0 and 120 minutes after the steady state level of hyperglycaemia was reached. MAGE = mean amplitude of glycaemic excursions, SBP = systolic blood pressure, DBP = diastolic blood pressure.

6.4.3 Glucose variability and biochemical analysis (II)

Positive univariate correlations appeared between HbA_{1c} and MAGE, mean daily glucose concentrations, and AUC+10 (Table 7). In addition, mean daily glucose concentrations correlated positively with total cholesterol and LDL-cholesterol. AUC-5 correlated negatively with LDL-cholesterol and positively with HDL-cholesterol.

The relationships between mean blood glucose and total cholesterol (r=0.42;*P*<0.05) as well as LDL-cholesterol concentrations (r=0.42;*P*<0.05) were independent of BMI, HbA_{1c}, or duration of diabetes in multivariate regression analysis. The correlation between AUC-5 and LDL-cholesterol (r=-0.47;*P*<0.05) was also independent.

Table 7. Univariate correlation coefficients between a continuous glucose monitoring system and biochemical analysis.

	AUC+10 (>10mmol/l)	AUC-5 (<5mmol/l)	MAGE	Mean daily glucose
HbA_{1c}	0.64 [a]	0.02	0.48 [b]	0.52 [a]
Total cholesterol	0.21	-0.39	-0.10	0.43 [b]
LDL-cholesterol	0.19	-0.45 [b]	-0.11	0.56 [b]
HDL-cholesterol	-0.04	0.47 [b]	0.19	-0.10
Triglycerides	0.17	-0.31	-0.06	0.16

[a] P< 0.01 and [b] P< 0.05. AUC+10 = area under the curve for glucose values exceeding 10 mmol/l, AUC-5 = area under the curve for glucose values below 5 mmol/l, MAGE = mean amplitude of glycaemic excursion, LDL = low-density lipoprotein, HDL = high-density lipoprotein, eGFR = estimated glomerular filtration rate.

6.5 Pre-eclampsia and diabetic nephropathy

6.5.1 Clinical characteristics of women with type 1 diabetes followed during pregnancy (V)

No clear differences appeared in baseline characteristics between patients participating in the follow-up study and those not participating. Clinical characteristics are shown in Table 2. The average follow-up time from the pregnancy to the follow-up visit was 10.6 ± 2.5 years. Patients with pregnancy-induced hypertension had a higher BMI than patients with uncomplicated pregnancies. Women with pre-eclampsia were younger and more often nulliparous than were patients in the other groups.

6.5.2 Pre-eclampsia and diabetic complications (V)

Women with pre-eclampsia were more likely to have diabetic nephropathy at follow-up than women with an uncomplicated pregnancy (41.9% vs. 8.9%, P<0.001, Table 2). Women with a history of pre-eclampsia had a higher frequency of coronary heart disease (12.2% vs. 2.2%, P<0.05). The same trend was observed with the number of patients on antihypertensive treatment at follow-up (50.0% vs. 9.8%, P<0.001).

Pre-eclampsia (p<0.001) and HbA_{1c} (all three trimesters) (p<0.05) during pregnancy were independently associated with diabetic nephropathy after adjustments for age, duration of diabetes, smoking, BMI, and follow-up time (Table 8).

Table 8. Logistic regression analysis for diabetic nephropathy.

	adjusted OR	95% CI	P-value
PE [a]	7.7	1.6-36.1	0.01
HbA_{1c} I trimester [b]	3.2	1.3-7.9	0.01
HbA_{1c} II trimester [b]	4.0	1.7-9.8	0.002
HbA_{1c} III trimester [b]	2.0	1.1-3.8	0.03

[a] Adjusted for BMI, follow-up time, smoking, duration of diabetes, age, and the average of the HbA_{1c} measurements during all 3 trimesters.
[b] Adjusted for BMI, follow-up time, smoking, duration of diabetes, and age.

6.5.3 Pregnancy-induced hypertension and complications (V)

Patients with pregnancy-induced hypertension were more often on antihypertensive treatment (41.9% vs. 9.8%, p<0.001, Table 2) than those with normal blood pressure during pregnancy. These patient groups did not differ with regard to diabetic nephropathy at follow-up (10.3% vs. 8.9%, NS).

6.5.4 Pregnancy characteristics and outcome (V)

Higher HbA_{1c} levels during pregnancy were associated with diabetic nephropathy (p<0.001). A large difference between HbA_{1c} during pregnancy and at follow-up predicted nephropathy at follow-up, supporting the role of glucose exposure as a risk factor for diabetic nephropathy.

7 Discussion and Conclusions

The novel findings of this thesis show that acute hyperglycaemia causes arterial stiffness both in patients with uncomplicated type 1 diabetes and non-diabetic subjects. The hyperglycaemia-induced inflammatory response may in part explain this finding. An acutely elevated blood glucose concentration leads to impaired ventricular repolarisation associated with sudden death. Furthermore, high mean daily blood glucose but not glucose variability *per se* is associated with arterial stiffness. While glucose variability in turn correlates with the central blood pressure, these results suggest that the glucose metabolism is closely linked to the haemodynamic changes in young patients with type 1 diabetes. Finally, a history of a pre-eclamptic pregnancy turned out to be associated with increased risk for diabetic nephropathy.

7.1 Limitations of the study

Several different methods and patient populations were employed in this series of studies. The stiffness of the arteries was measured with applanation tonometry. This method has been shown to be valid, and reproducible for the purpose. It enables measurement not only of the AIx but also the PWV. Importantly, aortic PWV has recently been considered the "gold standard" of arterial stiffness [212]. Moreover, PWV is a predictor of cardiovascular disease [189]. However, the PWV is limited by the fact that it measures the elastic properties of a single arterial segment and not the entire arterial system. Aix, in contrast, is a reproducible measure of generalized arterial stiffness, but to avoid inaccuracy has to be corrected for heart rate [279].

Regarding number of patients, the study sample in Studies I to IV was large enough to reveal a statistical significance between and within the studied groups. A power analysis was performed prior to the study and showed that the chosen number of patients was sufficient.

It is of note that because supraphysiological levels of insulin reduce arterial stiffness [112], any effect on the vasculature by hyperglycaemia could theoretically be mediated by a concomitant increase in insulin. In these studies, the insulin excretion of the healthy volunteers was blocked by somatostatin to control for any insulin effect. Moreover, other hormones that may also have affected results are glucagon, catecholamines, cortisone, growth hormones, and prolactin. However, HR did not increase during the clamp with regard to the catecholamines. Furthermore, a somatostatin analogue can reduce arterial stiffness in patients with acromegaly [280]. No studies regarding the effect of glucagon, known to be elevated in patients with type 1 diabetes, were available.

Only males were studied to avoid any hormonal variations that might have had an effect on arterial stiffness; results may therefore not be directly applicable to females. In addition, smokers, patients with diabetic complications, or those using any medication other than insulin were excluded.

Exclusion of these confounding factors made it possible, however, to explore independent effect of the glucose metabolism on the vasculature.

Regarding Study V, a few limitations must be acknowledged. Firstly, microalbuminuria, not measured quantitatively during pregnancy, could have been present in those patients with type 1 diabetes that did not progress to DN after pre-eclampsia. Proteinuria was, however, screened by the dipstick test, and an albuminuria greater than 0.3 g during early pregnancy could thus be excluded. The patients who had a positive dipstick test did in addition collect a 24 h urine sample to confirm their albuminuria status. Secondly, approximately 50% of the patients studied during their pregnancies did not attend the follow-up. It is noteworthy that these patients did not differ regarding their baseline clinical characteristics from those who did participate (data not shown). Thirdly, data from the time between the pregnancy and the follow-up visit were lacking; hence, a possibility exists that the patients with pre-eclampsia may have had poor glycaemic control after pregnancy, a fact that could have elevated their risk for diabetic nephropathy. This was, however, taken into account in the analyses by adjusting for level of glycaemia. That the odds ratio in the logistic regression analyses was as high as 7.7 after adjustment for glycaemia suggests that pre-eclampsia is a true predictor of diabetic kidney disease in patients with type 1 diabetes.

7.2 Acute hyperglycaemia and arterial stiffness

Type 1 diabetes and arterial stiffness are important risk factors for cardiovascular disease. Study I tested the hypothesis whether acute hyperglycaemia causes increased arterial stiffness and demonstrated that acute hyperglycaemia does cause increased arterial stiffness measured by AIx both in patients with type 1 diabetes and in healthy control subjects. These results confirm previous data by Mullan et al. and also further extend the observation to patients with type 1 diabetes [8].

This observation is also consistent with earlier findings in patients with type 2 diabetes showing depressed endothelial function during an oral glucose tolerance test [91]. These investigations by Kawano et al. were performed by measurement of flow-mediated endothelium-dependent vasodilation by an ultrasound technique. Notably, endothelial dysfunction is a key component of arterial stiffness, and thus these data from the Kawano group mirror the results seen in our study. Furthermore, a study by Williams et al. also showed similar results in healthy subjects, although they measured endothelial function by plethysmography [92]. Taken together, these data suggest that acute hyperglycaemia has a profound effect on the vasculature, and therefore it was not unexpected that Capes et al. suggest a link between acute hyperglycaemia and a worse prognosis after cardiovascular events, both in diabetic and non-diabetic patients [6]. Interestingly, similar results have also been reported after traumas in those non-diabetic [74].

Stiffness in intermediate-sized arteries (brachial PWV) in patients with type 1 diabetes increased, whereas no corresponding response occurred in healthy subjects. In contrast, the stiffness of large arteries (aortic PWV) did not change in response to acute hyperglycaemia in either group.

Speculatively, the discrepancy might be due to differences in the structure of the aorta and the brachial artery. The aortic walls consist mainly of elastin and collagen fibres, whereas the wall of the brachial artery contains a considerable number of smooth muscle cells. This observation may imply that chronic hyperglycaemia (diabetes) leads to increased reactivity to high glucose in small and intermediate sized arteries.

We know from earlier studies that arterial stiffness measured by pulse pressure is higher in patients with type 1 diabetes [228]. A few studies have demonstrated that the AIx is significantly higher in patients with type 1 diabetes than in healthy control subjects [199 203]. However, smokers and patients with hypertension and diabetic complications such as nephropathy were not excluded, a fact that might explain the increased stiffness. In the present study, in a homogenous young group of patients with type 1 diabetes and no complications, the presence of an increased arterial stiffness was confirmed and registered as a difference in Aix, but the study did not reveal any differences in either brachial or aortic PWV between diabetic and control subjects at baseline. Notably, these results are to some extent consistent with those from McEniery et al. which indicates that AIx may be a more sensitive marker of cardiovascular risk in younger individuals, whereas PWV may be a better measure in older subjects [281].

7.3 Glucose variability and haemodynamic variables

The use of the continuous glucose monitoring system has opened a new line in research through which it is possible to observe daily glucose variations in vivo. The measurements not only show glucose concentration that can be monitored with conventional methods but also show unpredictable glucose excursions. The most important characteristic of the CGMS is that it recognizes intra-day glucose variability, a phenomenon that is less studied. The results of the present study showed marked differences in glucose variability between patients (data not shown).

Study II showed that daily mean glucose concentration correlated with arterial stiffness but glucose variability did not. This study was thus among the first to provide data regarding glucose variability and surrogate markers for macrovascular disease in diabetes. Kilpatrick et al. [10] suggested that glucose variability does not predict microvascular complications in patients with type 1 diabetes. In another very recent paper, his group showed that daily mean blood glucose but not glucose variability predicted macrovascular disease [10 11]. Their results from the DCCT study were consistent with those from our study, but elicited a lively debate, and further studies were requested [282 283]. Interestingly, daily glucose fluctuations have been suggested to be associated with oxidative stress in type 2 but not in type 1 diabetes [166 167]. Perhaps the postprandial hyperglycaemia in type 2 diabetes is actually different from (and more atherogenic) than the glucose spikes seen in type 1 diabetes. Further studies are certainly needed to gain additional information [284].

Although mean blood glucose was associated with aortic PWV in Study II, Study I did not show an increase in aortic PWV in response to acute hyperglycaemia. These somewhat surprising results

may be explained by the fact that Study I demonstrated the effects of short-term (≤2 hours) hyperglycaemia, while Study II reflected mean blood glucose concentration over 3 days. This thesis study cannot, however, provide a definite answer to this question.

The findings of Study II may support the role of hyperglycaemia as an additional risk factor for macrovascular disease in patients with type 1 diabetes. The observations are also similar to those in the DCCT/EDIC where strict glycaemic control reduced the progression of coronary artery calcification, a surrogate marker of cardiovascular disease [62]. Orchard et al. [285] presented an interesting "glucose stabilization" theory proposing that glycaemia relates more strongly to the chronic stable atherosclerotic changes in peripheral arterial disease than to the unstable plaques in coronary arteries. Interestingly, he also suggested that the stable plaques in the peripheral arteries are related to the formation of AGEs [286]. In general, acute coronary events occur via plaque ruptures, and the plaques have been reported to be more vulnerable in patients with diabetes.

Another rather peculiar finding in this series was that patients with daily episodes of hypoglycaemia had more elastic arteries and also a more anti-atherogenic lipid profile. It can be speculated that these observations were due to better overall glycaemic control, an effect of the insulin or possibly of more frequent physical activity in these patients [287 288].

Results from the hyperglycaemic clamp demonstrated that patients with type 1 diabetes and with frequent daily glucose fluctuations had increased hyperglycaemia-induced haemodynamic reactivity. These observations imply that the vasculature does not habituate to hyperglycaemia. Rather, the opposite relationship was apparent. If these data are confirmed by other studies, they may have important clinical implications. SMBG should be intensively measured, regardless of HbA_{1c} concentration.

7.4 Acute hyperglycaemia and disturbed myocardial repolarisation

A prolonged QTc interval is a predictor of sudden death. Study III showed that acute hyperglycaemia prolongs the QTc interval both in patients with type 1 diabetes and in healthy volunteers. Cardiac arrest is, in fact, associated with QTc interval prolongation after an acute myocardial infarction, and since acute hyperglycaemia has been demonstrated to induce electrophysiological alterations in connection with an AMI, hyperglycaemia may be one of the factors that lead to arrhythmias [75].

Children and adolescents with type 1 diabetes have higher mortality than do non-diabetic subjects of the same age [289]. This observation originates from data linking these deaths to nocturnal hypoglycaemia ("dead-in-bed" syndrome) [290]. In this respect it is noteworthy that hypoglycaemia

also prolongs the QTc in type 1 diabetes [291], suggesting that both hyper- and hypoglycaemia disturb cardiac repolarisation.

Increased blood pressure, female sex, chronic hyperglycaemia (HbA_{1c}), genetic suspectibility, and ischaemic heart disease have been risk factors for QTc prolongation. In patients with type 1 diabetes, autonomic neuropathy and diabetic nephropathy are associated with a prolonged QTc interval [233]. Considering Study III, it appears that in patients with type 1 diabetes not only chronic but also acute hyperglycaemia is an additional risk factor for a prolonged QT interval [76]. Whether this explains the accentuated mortality in these patients during a cardiovascular event remains unknown.

Clearly, no definite conclusions on the pathophysiological mechanisms can be drawn based on Study III. It has been speculated that a prolonged QTc interval during hyperglycaemia may be due to an elevated cytosolic calcium content in the myocytes and a lower threshold for ventricular fibrillation and sudden death [292]. Thus, an acutely elevated blood glucose concentration may result in a decrease in the extracellular calcium concentration and an increase in the calcium influx into the cells, and consequently in a prolonged QTc interval.

Interestingly, the changes in repolarisation during hyperglycaemia have been reversed by inhibition of oxidative stress or of endothelial dysfunction in rats [238 239]. Increased sympathetic activity in response to acute hyperglycaemia is also possible [293], although Study III did not show increased heart rates during the clamp. Finally, disturbances in electrolyte balance may serve as an alternative explanation, since not only hypokalemia but also hypocalcemia can cause QT-interval prolongation.

7.5 Inflammatory changes in the vasculature during acute hyperglycaemia

The main finding of Study IV was that acute hyperglycaemia caused a response in the inflammatory markers both in young patients with type 1 diabetes without complications and in healthy age-matched control subjects. A hyperglycaemia-induced rise in the marker of oxidative stress also occurred.

To our knowledge, this may be is the first study to show an inflammatory response to acute hyperglycaemia in patients with type 1 diabetes. These data are, however, in line with the observations both from in vitro studies in monocytes and in vivo studies in non-diabetic subjects that acute hyperglycaemia indeed induces an increase in inflammatory cytokine concentrations [89 294]. Moreover, the increase in superoxide dismutase, a marker of oxidative stress, is also consistent with similar data by Marfella et al [101]. Finally, thus far unpublished data from the same series showed no increase in the cellular adhesion molecules ICAM and VCAM in response to acute hyperglycaemia. This is of course not unexpected, since the half-life of these molecules is very short, and a potential increase difficult to capture. We measured these molecules only at baseline

and after 120 minutes of hyperglycaemia, so any short-term increase between these time-points would have been missed.

Atherosclerosis is associated with chronic inflammation [82], and IL-6 and CRP are therefore not unexpectedly elevated in patients with cardiovascular disease [295]. While patients with type 1 diabetes have frequent episodes of hyperglycaemia as well as a substantially increased risk for cardiovascular disease and other diabetic complications, it can be speculated that hyperglycaemia (both chronic and acute) can induce inflammation (both chronic and acute), and this phenomenon may even serve as an additional mechanism for long-term diabetic complications. This view is in line with our previous data showing that patients with type 1 diabetes and diabetic nephropathy have elevated CRP and IL-6 concentrations as a sign of chronic inflammation [126].

An interesting theory has been presented by Blake et al., proposing that an inflammatory process disturbs the endothelial function of the arteries and thereby activates cellular adhesion molecules. These in turn attract circulating leucocytes to migrate into the subendothelial space. Consequently, macrophages express receptors for lipoproteins, forming lipid pools in the arteries. Furthermore, endothelial cells as well as smooth muscle cells start to express inflammatory cytokines, e.g., TNF-α, IL-1, and IL-6, stimulating the liver to produce CRP. Eventually the immunomodulator CD40 is expressed and mediates the expression of metalloproteinases; this disturbs the elastin/collagen synthesis and breakdown balance, possibly resulting in increased arterial stiffness as well as in thrombosis [296]. Another hypothesis is that the inflammatory response to acute hyperglycaemia may lead to ischaemia in the vessels of the arteries (vasa vasorum), and consequently to arterial stiffening [297].

Chronic hyperglycaemia leads to oxidative stress, a phenomenon essential for the development of diabetic complications. Acute hyperglycaemia also may cause oxidative stress in non-diabetic subjects [101], an observation confirmed by Study IV. Antioxidants have in experimental studies reduced the adverse effects of acute hyperglycaemia on endothelial function and inflammation [298]. Further studies are, however, needed to clarify the role of antioxidant therapy in the prevention of cardiovascular disease.

7.6 Pre-eclampsia and diabetic nephropathy

The most important finding of Study V was that women with both type 1 diabetes and pre-eclampsia during pregnancy were much more likely to develop diabetic nephropathy and high blood pressure later in life than were women with type 1 diabetes and normal blood pressure during pregnancy. Pregnancy-induced hypertension did not predispose to subsequent diabetic kidney disease at follow-up, suggesting that these two entities (pre-eclampsia and pregnancy-induced hypertension) truly represent different diseases.

A few studies have shown that pre-eclampsia predicts cardiovascular disease in the general population [299]. Study V confirms that this is also true for women with type 1 diabetes. To our knowledge, only one study has explored the association between pre-eclampsia and microvascular disease; it suggested that pre-eclampsia is associated with diabetic retinopathy [255].

Due to lack of a control group, Study V could not answer the question whether non-diabetic women with pre-eclampsia also are at higher risk for kidney disease. However, in an earlier study from our group, pre-eclamptic women without diabetes were followed for 5 years, and none of them showed any signs of kidney disease at follow-up [300].

The mechanisms for these findings may relate to the endothelial dysfunction or low-grade inflammation present both in nephropathy and pre-eclampsia [126 301 302 303]. However, at the morphological level, pre-eclampsia seems to be quite different from diabetic nephropathy. Pre-eclampsia is more of a focal glomerulonephritis [304], while nephropathy is characterized as general glomerulosclerosis [305]. One could speculate that pre-eclampsia may be a trigger and thus cause an insult to the kidney that predisposes to nephron loss and further to diabetic nephropathy in susceptible individuals.

Interestingly, there may emerge a common genetic background for pre-eclampsia and diabetic nephropathy, possibly through endothelial dysfunction, chronic inflammation, or insulin resistance [306]. Another possibility is that pregnancy itself as a state of hypervolemia, acquired thrombophilia, insulin resistance, and low-grade inflammation could induce pre-eclampsia as the first manifestation of endothelial dysfunction in genetically susceptible individuals [307]. Most likely several different factors play roles in the relationship between these two diseases.

7.7 Summary and conclusions

Acute hyperglycaemia was found to increase arterial stiffness in both males with type 1 diabetes and in healthy age-matched non-diabetic male volunteers. A similar response to an acutely elevated blood glucose concentration was observed in myocardial ventricular repolarisation, which was prolonged in both groups. Inflammation and activation of oxidative stress also turned out to be involved in the process. Notably, male patients with fluctuating daily blood glucose concentrations showed an increased response to acute hyperglycaemia with respect to their central blood pressure. On the other hand, a high mean daily blood glucose concentration was associated with increased arterial stiffness, whereas HbA_{1c} was not associated with arterial stiffness. Finally, women with type 1 diabetes and pre-eclampsia during their pregnancy developed diabetic nephropathy more often than did those with uncomplicated pregnancies. These findings underline the importance of strict glycaemic control in patients with type 1 diabetes as a means to avoid cardiovascular complications.

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